

SOME DETERMINANTS OF HEAT PRODUCTION CAPACITY
AND SUSCEPTIBILITY TO HYPOTHERMIA IN NEWBORN LAMBS

by

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DECLARATION

This thesis was composed by the undersigned who, subject to the acknowledgements made above, was also responsible for the research work described herein. This work was carried out at the Animal Diseases Research Association, Moredun Research Institute, Gilmerton, Edinburgh, under the supervision of Dr D.J. Mellor and Professor W.E. Watson.

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ABSTRACT

Hypothermia is caused by an imbalance between the heat produced by a lamb and the heat lost from it to the environment. At the beginning of this work the determinants of heat loss were well established but the determinants of heat production were poorly understood. This thesis describes first a laboratory investigation of some determinants of heat production capacity in newborn lambs and secondly, a field investigation of the causes of hypothermia.

Heat production capacity was estimated as summit metabolic rate and a new technique was devised to elicit this state. Summit metabolic rate was measured in two breeds of lambs aged up to five hours. Similar rates were recorded in Scottish Blackface and in Dorset Down lambs. Full thermogenic capacity was achieved within one hour of birth. Exposure to severe hypoxia either during birth or after birth resulted in a subsequent temporary depression of summit metabolic rate, in some cases to less than 50% of the control value. This depression was associated with a low blood pH and high plasma levels of catecholamines. Summit metabolic rate was best related to body weight raised to the power of 0.75. Carbohydrate appeared to be the major energy substrate during summit metabolism. Feeding with colostrum in the first three hours after birth resulted in a 20% increase in summit metabolic rate at five hours of age.

Clinical cases of hypothermia on commercial farms were examined in terms of history, biochemistry and pathology in order to define the causes of the condition and any predisposing factors. Two major causes were identified; excessive heat loss from the wet newborn lamb aged up to five hours and depressed heat production due to starvation in

lambs aged 12 hours or more. A lamb was more susceptible to hypothermia if it had suffered severe hypoxia during birth, was immature or was a twin or triplet. In parallel with this investigation, new and more effective techniques for the detection and treatment of hypothermia were developed. These comprised a simple to use electronic thermometer, the parenteral administration of glucose to hypoglycaemic lambs, rewarming in warm air and careful after-care. These techniques were subjected to extensive field trials and have been widely adopted.

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SUMMARY

The work described in this thesis was undertaken at the Moredun Research Institute in Edinburgh in an attempt to improve the understanding of the problem of hypothermia in newborn lambs which annually causes the death of about one million lambs. A chapter by chapter summary is presented below to give the reader an outline of the whole work before proceeding to the chapters themselves.

CHAPTER ONE - INTRODUCTION

Hypothermia occurs when the heat loss from a lamb exceeds its rate of production. The rate of heat loss is related to the insulation value of the fleece which is reduced by wetting, to climatic conditions including environmental temperature and wind, and to the mothering ability of the ewe. These factors determining heat loss were well understood and were not investigated further in the laboratory.

In contrast, the determinants of heat production capacity in newborn lambs were poorly understood and the sources of considerable between-lamb variation were not clear. A substantial part of this thesis describes an investigation of this variation. Heat production capacity was estimated as summit metabolic rate - the highest metabolic rate which can be induced by exposure to cold stress.

On the basis of the results of this investigation and the published work of others, a field investigation of the causes of hypothermia was undertaken. In parallel with this work, new techniques for the detection and treatment of hypothermia were developed.

CHAPTER TWO - MATERIALS AND METHODS

Lambs of two breeds, Dorset Down and Scottish Blackface were used. Management before, during and after lambing was according to normal sheep husbandry practice as far as experimental requirements permitted.

Metabolic rate was measured by indirect calorimetry. Basal metabolic rate was estimated during immersion of the lamb's body in water at 38.5°C and summit metabolic rate was elicited by a progressive cooling of the water until rectal temperature started to fall.

During laboratory investigations blood samples were taken by a catheter in the vena cava inserted via an umbilical vein and in field investigations by needle puncture of a jugular vein. The treatment, storage and analysis of samples is described in detail.

CHAPTER THREE - HEAT PRODUCTION

Basal metabolic rate was estimated in Scottish Blackface lambs aged either 1½ or 4½ hours. The rates recorded were similar to those obtained by other workers, and this finding together with the very quiet behaviour of the lambs during the estimations, indicated that the water immersion and indirect calorimetry procedures did not themselves impose any stress on the lambs, and that any increase in metabolic rate which might be observed during cold stress would be primarily attributable to this stimulus.

Summit metabolic rate was estimated in Scottish Blackface lambs aged either two or five hours. Considerable lamb to lamb variation in summit metabolic rate was identified and a substantial part of this variation was attributed to severe hypoxia during birth. Lambs which showed evidence of severe hypoxia, severe metabolic acidosis

and hyperlactaemia after birth, had low summit metabolic rates. During cooling, respiratory quotient first decreased but then increased to mean values of 0.90 (two-hour old) and 0.94 (five-hour old) during summit metabolism, suggesting that carbohydrate was the major energy substrate at this time. Cooling was associated with substantial increases in the plasma levels of glucose, free fatty acids and glycerol and the development of a metabolic acidosis and hyperlactaemia. There were marked increases in the plasma levels of catecholamines and corticosteroids and a marked decrease in the plasma insulin level. The increase in metabolic rate from basal metabolism to summit metabolism was positively correlated with the associated increases in the plasma concentrations of glucose, lactate, free fatty acids and glycerol and negatively correlated with the changes in blood pH and base excess. These correlations suggested that tissue oxygen supply might have been a factor which limited metabolic rate in lambs with high rates of summit metabolism whereas tissue energy substrate supply might have limited metabolic rate in lambs which showed lower rates.

In an attempt to further investigate the relationship between hypoxia during birth and heat production capacity, summit metabolic rate was estimated in lambs aged one hour on the assumption that any deleterious postnatal effects of severe hypoxia during birth would be most evident immediately after birth. In the group of lambs studied no cases of severe hypoxia were encountered and no relationship between summit metabolic rate and indices of hypoxia could be established. This experiment did however demonstrate that mild hypoxia during birth, which commonly occurs, has no effects on heat production at one hour of age.

At this stage of the investigation an analysis was conducted of the relationship between summit metabolic rate and body weight. This analysis was performed for each of three groups of lambs which were aged one, two and five hours. Age had no significant effect on the relationship and thus the analysis was repeated using all the data. Summit metabolic rate was best related to body weight raised to the power 0.75. This exponent was not significantly different from either 1.00 (summit metabolic rate directly proportional to body weight) or 0.59 (summit metabolic rate directly proportional to body surface area, which is a determinant of the rate of heat loss from a lamb).

In order to examine further the relationship between heat production capacity and prior exposure to severe hypoxia, summit metabolic rate was estimated after exposure to postnatal hypoxia. Lambs were exposed to hypoxia for either 30 minutes or up to three hours. In both cases the severity of the hypoxia was adjusted to produce a metabolic acidosis at the end of the hypoxia with a base excess value of -20m equiv./l. Summit metabolic rate was estimated either as soon as possible after the end of the period of hypoxia or after a 30 minute recovery period. Summit metabolic rate immediately after a short period of hypoxia was depressed to 66% of the control value but no depression was evident after a 30 minute recovery period. After a long period of hypoxia the 'immediate' summit metabolic rate was depressed to 62% of the control value and after a 30 minute recovery period to 80% of the control value. In addition to a metabolic acidosis, exposure to hypoxia resulted in a hyperlactaemia and a marked elevation of the plasma catecholamine levels. During the recovery period there was a partial reversal of all these changes in blood composition. Little depression of summit metabolic rate was observed

in lambs which had a blood pH value at the beginning of summit metabolism of 7.05 or greater and in these lambs there were no relationships between blood composition at this time and summit metabolic rate. However, in lambs showing blood pH values of less than 7.05 summit metabolic rate was positively correlated with the blood pH value and negatively correlated with the plasma adrenaline level. Either the low blood pH, the high adrenaline level or both these factors could have been responsible for the depression of summit metabolic rate.

The effects of feeding colostrum between one and three hours after birth were examined in lambs aged five hours. Feeding resulted in a 17-20% increase in summit metabolic rate. Higher plasma glucose and insulin levels in the fed lambs suggested that an enhanced supply of glucose to the heat producing tissues was responsible for this increase.

CHAPTER FOUR - CAUSES OF HYPOTHERMIA

Whilst there has been extensive study of the physiology of temperature regulation in the newborn lamb, there has been little investigation of the causes of hypothermia in the field. This chapter describes such an investigation.

In the first stage 89 cases of hypothermia were examined in terms of history, physical condition and clinical chemistry. Postmortem examinations were performed on 15 of the lambs which were not successfully treated. The causes of hypothermia were diagnosed as excessive heat loss (38%), depressed heat production due to severe hypoxia during birth (10%), depressed heat production due to starvation (47%) and other causes (5%). Hypothermia resulting from excessive heat loss and depressed heat production due to severe hypoxia during birth

occurred mostly in the first eight hours of life. Hypothermia resulting from depressed heat production due to starvation occurred in lambs aged eight hours or more. Immaturity was identified as a factor contributing to the hypothermia in 35% of the cases.

In the second stage of this study less detailed records were kept of 493 hypothermic lambs on 30 farms. The condition was more common in twin and triplet lambs than in singles.

CHAPTER FIVE - DETECTION AND TREATMENT OF HYPOTHERMIA

The work described in this chapter was conducted in parallel with that described in the previous chapter. A regime for the detection and treatment of hypothermia was developed. This regime comprised four components:

1. the early detection of hypothermia with the aid of a thermometer;
2. the parenteral administration of glucose solution to reverse hypoglycaemia in starving lambs;
3. warming in air at 40°C;
4. care after warming.

An extensive field trial of this regime was conducted using a novel electronic thermometer to aid early detection. Very satisfactory results were recorded; 69% of all lambs treated were alive one week after treatment. Early detection of hypothermia considerably enhanced the chances of success.

The techniques and equipment described in this chapter have been further developed and are now accepted in commercial practice.

TABLE OF ABBREVIATIONS

kg	kilogram
g	gram
mg	milligram
µg	microgram
m	metre
cm	centimetre
mm	millimetre
µm	micrometre
nm	nanometre
ft	foot
l	litre
ml	millilitre
µl	microlitre
h	hour
min	minute
°C	degree Celsius
MJ	megajoule
kJ	kilojoule
kcal	Calorie (4.2 kJ)
W	watt
kW	kilowatt
kPa	kilopascal
M	mole per litre
mM	millimole per litre
µM	micromole per litre
nM	nanomole per litre
pM	picomole per litre
m eq (equiv)	milliequivalent

pH	negative log of the hydrogen ion concentration in gram ions per litre
PO ₂	partial pressure of oxygen
PCO ₂	partial pressure of carbon dioxide
PvO ₂	partial pressure of oxygen in venous blood
PvCO ₂	partial pressure of carbon dioxide in venous blood
NAD	oxidised nicotinamide-adenine dinucleotide
NADH	reduced nicotinamide-adenine dinucleotide
ATP	adenosine triphosphate
ADP	adenosine diphosphate
IgG	immunoglobulin G
T ₃	tri-iodothyronine
n	number of items in a sample
SD	standard deviation
SEM	standard error of the mean
r	coefficient of correlation
P	probability
*	$P < 0.05$
**	$P < 0.01$
***	$P < 0.001$
NS	not significant
BW	body weight
MR	metabolic rate
BMR	basal metabolic rate
SMR	summit metabolic rate

CHAPTER I

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INTRODUCTION

The work described in this thesis was conducted at the Moredun Research Institute of the Animal Diseases Research Association. The remit of this Institute, which is mainly supported by public funds, is the investigation of disease in farm animals, mainly sheep and cattle. One condition which causes a considerable loss in the sheep industry is hypothermia in newborn lambs.

In the United Kingdom there are approximately 12 million breeding ewes (Goodwin, 1979) and each year these ewes produce approximately 15 million live lambs. The total rate of mortality in these lambs in the first week of life can vary from 2% to in excess of 30% (Meat and Livestock Commission 1976; Slee, 1976), the average loss being about 15%. Approximately half of these losses can be attributed to hypothermia, the remaining losses being accounted for by infectious disease, accidents and other causes (Houston and Maddox, 1974; Saunders, 1977, Johnston, 1977, Speedy, Linklater, MacKenzie, MacMillan and Blance, 1977).

The cost of lamb mortality to the sheep industry was reviewed by Howe in 1976. Correcting to 1983 prices the total annual cost of mortality from birth to weaning is approximately £30 million. Deaths in the first week of life account for at least 75% of these losses (Whitelaw, 1977). Hypothermia accounts for one half of these losses and thus this condition costs the industry at least £11 million each year. The cost of hypothermia to an 'average' sheep farmer with 500 ewes can be calculated to be about £1000.

Hypothermia occurs when the rate of heat loss from a lamb exceeds its rate of heat production. Thus in any consideration of this problem

it is necessary to evaluate the determinants of both heat production and heat loss.

1.1 HEAT LOSS

Heat is lost from a lamb via the skin and via the respiratory tract. Loss from the respiratory tract is obligatory and is of a sufficiently low order that it can be ignored in the context of hypothermia (Alexander, 1961). Heat loss via the skin depends on the temperature gradient between the skin surface and the environment, and on the insulation value of the fleece. As environmental temperature declines so also does skin temperature but to a lesser extent. At an environmental temperature of 40°C there is no temperature gradient between skin and environment, whilst at 10°C the skin to environment gradient is 15-20°C and at -10°C the gradient increases to 25-35°C (Alexander, 1961). This increase in gradient is associated with an increase in the rate of heat loss. A 5kg lamb in still air at 10°C loses heat at approximately 24Kcal/hour whereas at -10°C this rate is increased to 36Kcal/hour (Alexander, 1962a). The reduction in skin temperature in a cold environment is related to cutaneous vasoconstriction. When a lamb is transferred from a warm environment to a cold one skin blood flow may be halved (Alexander, Bell and Setchell, 1972). There is evidence to suggest that the capacity for cutaneous vasoconstriction in the lamb immediately after birth is less than that in the older lamb, since heat loss from such lambs exceeds that from wetted older lambs (Alexander, 1962a). Skin surface temperature is probably also related to skin thickness (Samson and Slee, 1981).

The insulation value of the fleece depends on the type of fleece, the effect of wind on the structure of the fleece and on the degree

of wetness. Both the fibre type and the length of the fleece influence the insulation value. Least heat is lost through a long hairy fleece and most through a short fine fleece (Alexander, 1962a; Purser and Karam, 1967; Slee, 1978). The density of secondary wool fibres is positively correlated with bodyweight and thus smaller lambs are likely to have fleeces of poor insulation value (Alexander, 1964). Wind reduces the insulation value of the fleece by disturbing the air trapped within it (Alexander, 1962a). The insulation value of the fleece is reduced by wetting to as low as 60% of the dry value (Fig. 1.1), the reduction being greater with fine coats than with hairy coats (Alexander, 1962a). A second factor increasing the rate of heat loss from a wet lamb is the evaporation of water from the coat. The efficiency of cooling is of the order of 25% (Alexander, 1962a). In other words 25% of the heat required to vaporise the water on the surface of the coat comes from the lamb. The rate of heat loss via evaporation from the wet coat is increased in windy conditions (Fig. 1.1). In this context it is significant that the highest losses from hypothermia in the field are recorded during wet windy weather (Moule, 1954; Watson, Alexander, Cumming, MacDonald, McLaughlin, Rizzoli and Williams, 1968; Alexander, Lynch, Mottershead and Donnelly, 1980).

In the field situation the rate of heat loss from a lamb is dependent on the climatic conditions, the availability of shelter and the behaviour of the ewe. The impact of the climate on the lamb can be considerably modified by the use of shelter such as walls, straw bales, or grass wind breaks (Alexander, Lynch, Mottershead and Donnelly, 1980) the major factor being a substantial reduction of wind speed. The most effective form of shelter is housing which not only abolishes wind but also prevents wetting of lambs and reduces to a

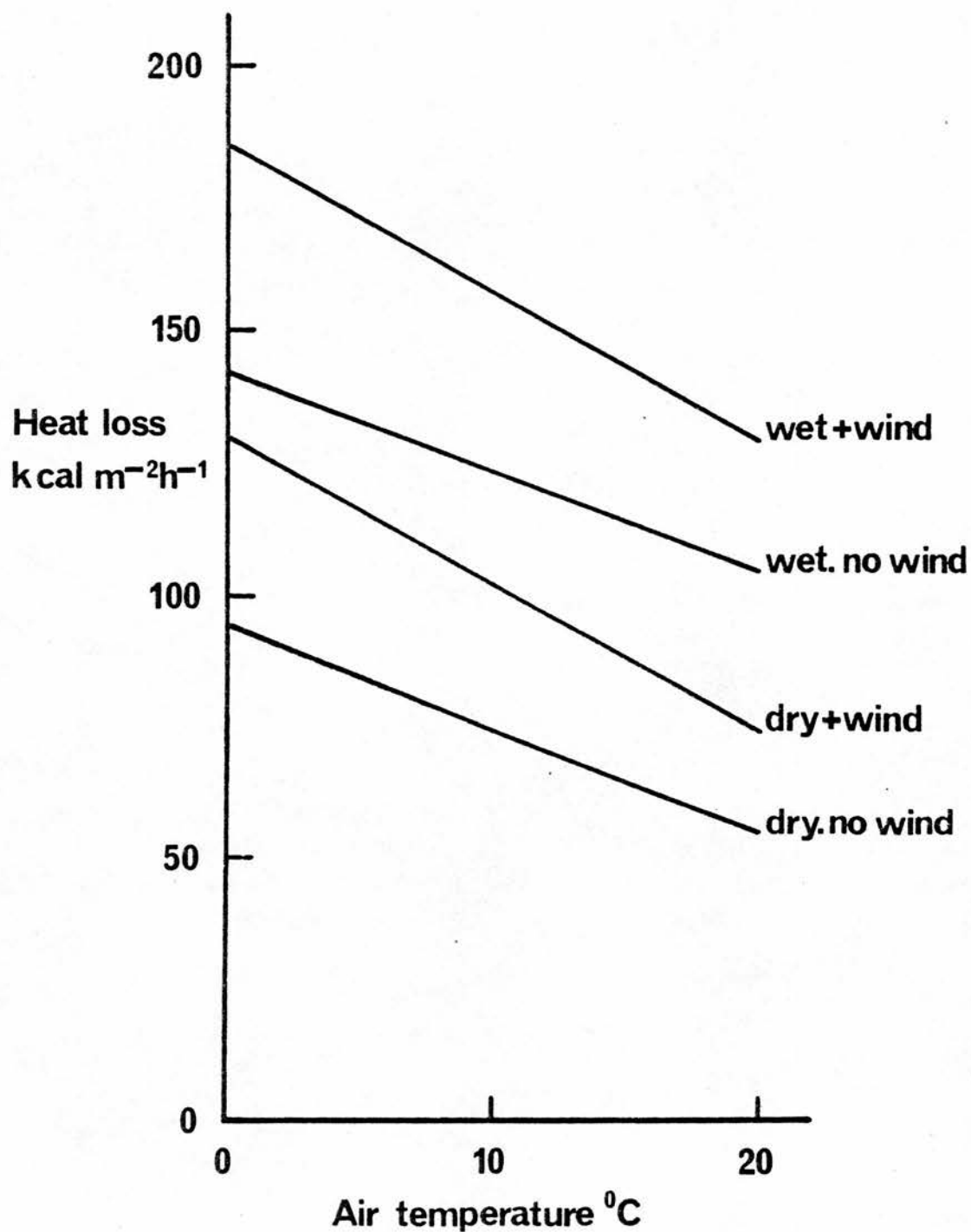


Figure 1.1 Heat loss due to wind and evaporation from lambs with hairy coats related to ambient temperature (drawn from Alexander, 1962a).

minimum evaporative loss from all except newborn lambs. The ewe can affect heat loss in two ways. First within two minutes of birth she commences to lick the lamb dry (Alexander and Peterson, 1961; Arnold and Morgan, 1975) and she may spend up to 50% of the first hour after birth in this activity (Bareham, 1976). Failure to do this inevitably results in a sustained high rate of heat loss. Secondly the ewe will lead the lamb to shelter and in addition will serve as a form of shelter herself (Fig. 1.2).

1.2 HEAT PRODUCTION

Heat production in all species has recently been comprehensively reviewed by Alexander (1979) and it is not the purpose of this section to duplicate this. However it is appropriate to examine the knowledge of heat production in the newborn lamb which laid the foundations for the work later described.

1.2.1 Assessment of Heat Production Capacity

There are two rates of heat production which can be accurately defined for a lamb of a particular age. These are the lowest rate or basal metabolic rate and the highest rate or summit metabolic rate. In the context of hypothermia basal metabolic rate has no direct practical significance but summit metabolic rate is an estimation of a lamb's ability to maintain its body temperature in conditions of high heat loss for a short period.

Heat production capacity can also be estimated in terms of the time for which a lamb can maintain homeothermy in a defined environment (Alexander, 1962c), in changes of the upper and lower critical temperature (Mercer, Andrews and Szekely, 1979) or in terms of the

Figure 1.2 Twin lambs being sheltered by their dam. The wind direction which was from 'top left' to 'bottom right' is indicated by the lie of the loose straw.



rate of temperature decline during acute cold stress (Slee, Griffiths and Samson, 1980). However, these estimations can be confounded by the effects of starvation, feeding or varying undefined rates of heat loss.

1.2.2 Mechanisms of Heat Production in Newborn Lambs

There are three mechanisms of heat production in the newborn lamb. These are basal metabolism, the obligatory rate of heat production under thermoneutral conditions, and shivering and non-shivering thermogenesis which are induced when the rate of heat loss exceeds the basal metabolic rate. In the newborn lamb shivering and non-shivering thermogenesis appear to be of approximately equal quantitative significance when the increase in metabolic rate from basal to summit metabolism is considered (Alexander and Williams, 1968). Shivering thermogenesis is an 'increase in the rate of heat production during cold exposure due to increased contractile activity of skeletal muscle not involving voluntary movements and external work' (Bligh and Johnson, 1973). Although shivering occurs in skeletal muscles it is an involuntary function (Alexander, 1979). The significance of shivering thermogenesis in the newborn lamb is reflected in the four-fold increase in blood supply to skeletal muscle when a lamb is exposed to cold (Alexander, Bell and Setchell, 1972). Shivering thermogenesis in the lamb has received little attention from investigators and there has been no suggestion that any substantial part of the variation between lambs in thermogenic potential is related to any restriction of shivering thermogenesis.

In contrast to shivering thermogenesis, non-shivering thermogenesis in the newborn lamb and in other species has received considerable attention. Non-shivering thermogenesis in the newborn lamb

is attributed to brown fat metabolism although it is possible that other mechanisms may be involved (Alexander and Williams, 1968).

Brown fat comprises 1-2% of the bodyweight of the newborn lamb (Alexander and Bell, 1975) and it is widely distributed throughout the body, major deposits being found in the peri-renal/abdominal region, the pre-scapular/cervical region, the inguinal region, the peri-cardial region and the lumbar region. As in other species brown fat metabolism is under the control of the sympathetic nervous system (Hull, 1966; Hull and Segall, 1965b; Alexander and Williams, 1968). Infusion of noradrenaline to lambs under thermoneutral conditions reproduces the changes in blood composition associated with cold exposure and causes an increase in heat production (Alexander, Mills and Scott, 1968; Alexander, 1969). Infusion with sympathetic blocking agents such as the beta blocker propranolol depresses the metabolic response to cold stress (Alexander and Williams, 1968; Alexander and Stevens, 1980). The contribution of brown fat metabolism to summit metabolism in lambs appears to decline with age from approximately 50% at birth to about 10% at one month (Alexander, 1979). The significance of brown fat metabolism to heat production in the newborn lamb is reflected in the five-fold increase in blood flow to this tissue which occurs when the newborn lamb is exposed to cold (Alexander, Bell and Hales, 1973).

The activity of brown fat is dependent on supplies of oxygen, lipid and carbohydrate (Hull and Segall, 1965b; Hull, 1966; Alexander, 1970; Cannon, Nedergaard, Romert, Sundin and Svartengren, 1978). The depletion of the fat content of brown adipose tissue during cold exposure suggests that intrinsic fat is utilised (Alexander, 1962c) but it seems probable that brown fat can also utilise lipid from

other sources otherwise the tissue would become practically inactive within a day or even hours of birth.

There has been considerable debate concerning the mechanism of heat production in brown fat. In 1965 a 'futile' cycle of triglyceride breakdown and resynthesis was proposed (Dawkins and Hull, 1965) but in recent years an uncoupling of oxidative phosphorylation with the conversion of the energy of substrate breakdown into heat instead of the synthesis of ATP has been advocated (Himms-Hagen, 1978; Alexander, 1979). A similar mechanism has been proposed for the uncontrolled increase in heat production observed when some pigs and some people are exposed to certain anaesthetic agents (Eikelenboom and Sybesma, 1974).

Chronic hypoxia in utero in the rabbit induced by the prolongation of gestation results in an in vivo depression of brown fat activity after birth (Harding and Ralph, 1970). There is no information on the activity of brown fat in newborn lambs which have suffered hypoxia in utero but an association between a prolonged parturition with acute hypoxia and a low summit metabolism immediately after birth has been suggested for the lamb (Alexander, 1962b).

1.2.3 Summit Metabolism

(a) Estimations in lambs. Most estimations of summit metabolism in newborn lambs are attributed to Alexander (Alexander, 1962a; Alexander, 1962b; Alexander and Williams, 1970). The mean value for summit metabolism calculated from 100 Merino and crossbred lambs aged up to 3 days was 20W/kg, approximately five times the basal metabolic rate. This value compared favourably with that observed in the adult sheep of 10W/kg (Bennett, 1972), in the newborn pig of 14W/kg (calculated from

Mount and Stephens, 1970) and in the newborn rabbit of 18W/kg (calculated from Hull and Segall, 1965a).

Probably the most important finding made by Alexander was not the mean rates of summit metabolism but the considerable lamb to lamb variation. Examination of individual values shows that some lambs produced twice as much heat as others (Alexander, 1969).

(b) Summit metabolism and body weight. Alexander (1962b) found that summit metabolism was closely related to body weight. Since body surface area is proportional to body weight raised to the power 0.59 (Pierce, 1934) summit metabolism per unit body surface area decreased as body weight decreased. This finding was suggested as the probable explanation for the higher rate of mortality from hypothermia in Australian small lambs since heat loss is directly proportional to body surface area. This finding has been extrapolated to the European situation (Slee, 1976). A similar relationship between summit metabolism and body weight has been found in the newborn pig (Mount and Stephens, 1970). In the adult sheep summit metabolic rate is proportional to body weight raised to the power 0.9 (Bennet, 1972).

(c) Summit metabolism and gestational age. Prematurity is associated with a low summit metabolic rate (Alexander, Thorburn, Nicol and Bell, 1972). Lambs born at 135 days of gestation following fetal adrenocorticotrophin infusion only produced a summit metabolic rate of 70% of the normal term value. At a conceptual age of 145 days these lambs still showed a subnormal summit metabolism. These observations help to explain the high rate of mortality observed in prematurely born lambs in the field (Dawes and Parry, 1965).

(d) Summit metabolism and postnatal age. Summit metabolism has been

found to decline with age (Alexander, 1962b; Alexander and Williams, 1970). When expressed on a body weight basis, summit metabolism at two months of age was found to be approximately 60% of the value obtained on the first day of life (Alexander and Williams, 1968). However, estimates based on this data show no decline in summit metabolism when expressed on a surface area basis. The situation in the newborn lamb contrasts with that in the newborn pig in which summit metabolism was found to increase after birth (Mount and Stephens, 1970).

(e) Summit metabolism and body temperature. There is no clear relationship between summit metabolic rate and rectal temperature at rectal temperatures exceeding 36°C (Alexander, 1962b) but below this temperature summit metabolic rate falls to an extrapolated zero between 20 and 25°C . Similar observations have been made in the adult sheep (Bennett, 1972).

(f) Summit metabolism and prenatal nutrition. Lambs from ewes fed poorly during pregnancy were found to cool more readily than those from well-fed ewes when exposed to cold stress (Alexander 1962a) but summit metabolism in these two classes of lambs was not found to differ when expressed on a body weight basis (Alexander, 1962b). However, lambs from poorly fed ewes tend to be smaller and thus have a lower rate of heat production per unit body surface area, assuming a linear relationship between summit metabolic rate and body weight. They would therefore have less ability to resist cooling.

(g) Summit metabolism and postnatal nutrition. Milk intake in the newborn lamb was associated with an increase in metabolic rate (Alexander, 1961) but this increase was not maintained during summit

metabolism (Alexander, 1962b), presumably because factors other than substrate supply to thermogenic tissues were limiting. Starvation of the newborn lamb led to an exhaustion of body energy reserves and death from hypoglycaemia and hypothermia in as short a time as 16 hours (Alexander, 1962c). Dehydration was not the cause of death. It can reasonably be assumed that starvation leads to a depression of summit metabolic rate.

(h) Summit metabolism and hypoxia and hyperoxia. Hypoxia (12-13% inspired oxygen) depressed summit metabolic rate whilst hyperoxia (33% inspired oxygen) only resulted in a small increase (Alexander and Williams, 1970). During cold stress there is a marked increase in the plasma concentration of lactate and a metabolic acidosis develops (Alexander, Bell and Hales, 1972) suggesting a degree of tissue hypoxia (Huckabee, 1958; Krebs, Woods and Alberti, 1975; Cain, 1977). It would appear that whilst supply of oxygen to the lungs does not normally limit summit metabolic rate transport of oxygen from the lungs to the thermogenic tissues may well do so.

1.2.4 Substrates for Heat Production

(a) Blood composition during cold stress. Blood and plasma substrate concentrations, blood gases and acid-base status during cold stress have been extensively reported and show similar changes to those found during strenuous exercise. Cold stress and a consequent increase in heat production are associated with increases in the plasma concentrations of glucose, free fatty acids, lactate and glycerol, suggesting that both carbohydrate and fat are important substrates for heat production (Van Duyne, Parker, Havel and Holm, 1960; Alexander, 1962b; Alexander, Mills and Scott, 1968; Alexander and Mills, 1968; Alexander, Bell and Hales, 1972). Summit metabolism in lambs which have not been

starved is not associated with an increase in either amino acid nitrogen or urea nitrogen (Alexander, 1962b) suggesting that protein catabolism is not a significant source of heat production when carbohydrate and lipid supplies are not limiting.

(b) Respiratory quotient during cold stress. Respiratory quotient was extensively measured by Alexander and his colleagues (Alexander, 1961; Alexander, 1962b; Alexander and Williams, 1968). However both the conditions under which respiratory quotient was measured and the results obtained were very variable. The least controlled condition was age. Although these workers generally considered that fat became an increasingly important substrate as metabolic rate increased in response to cold stress, they did record respiratory quotient values of between 0.87 and 0.91 during summit metabolism. What can be safely concluded is that respiratory quotient during cold stress in newborn lambs is related to metabolic rate, age and the duration of fasting.

(c) Body energy reserves. The fat content of the newborn lamb has been estimated at 2 - 3.4% of body weight and is related to both litter size and prenatal nutrition (Alexander, 1962c; Alexander and Bell, 1975). Carbohydrate reserves are found concentrated in the liver and in skeletal muscle and constitute approximately 1% of body weight at birth. There is no clear relationship between prenatal nutrition and carbohydrate reserves (Alexander, 1974). The protein content of the newborn lamb in terms of muscle alone must considerably exceed that of both fat and carbohydrate but this cannot be considered a readily available substrate store. The total calorific value of the fat and carbohydrate reserves of the newborn lamb (assumed to be 3 and 1% of body weight respectively) can be calculated to be approximately 1.3 MJ/kg. The rate of energy expenditure at summit metabolism is

approximately 0.1 MJ/kg per hour. Thus it can be calculated that theoretically the newborn lamb has energy reserves with which it can support heat production at summit metabolism for 13 hours.

(d) Milk. Ewe colostrum contains approximately 12% fat, 4% carbohydrate and 8% protein (see 3.5.3). If colostrum consumption is assumed to be 200 ml/kg in the first day of life (Shubber, Doxey, Black and FitzSimmons, 1979) the calorific intake attributable to fat and carbohydrate can be calculated to be about 1.3 MJ/kg, equivalent to 13 hours of energy expenditure at summit metabolism.

(e) Substrates for heat production during starvation. There has only been one study of heat production during starvation in which substrate utilisation was studied (Alexander, 1962c). In this study lambs were starved from birth at an environmental temperature of either 9 or 23°C. Death occurred within 23-68 hours. Survival time was longer in single lambs than in twins, in lambs which came from well-fed ewes and in lambs maintained at the higher temperature. During starvation the plasma concentration of glucose progressively declined to about 1mM just before death and this low glucose level together with hypothermia was the immediate cause of death. The plasma concentration of urea changed little during the first 12 hours of starvation but thereafter progressively increased. Carcass analysis after death indicated a depletion of body fat of about 50%. These observations suggested that fat, carbohydrate and protein were all substrates for heat production. Whilst fat was assessed to be quantitatively the most important substrate, it was the limited reserves of carbohydrate which determined survival time.

(f) The relative importance of carbohydrate, lipid and protein as substrates for heat production. All the evidence presented suggests

that protein is a relatively unimportant source of substrate for heat production in the newborn lamb. Even in the starved lamb, protein catabolism only became evident after 12 hours. The principal sources are carbohydrate and lipid.

There has been no detailed investigation of carbohydrate and lipid metabolism in the newborn lamb. In the foetus in late pregnancy carbohydrate appears to be the major energy source (Hodgson, Mellor and Field, 1979, 1980 and 1981). The newborn lamb can clearly use both substrates but cannot approach the position in the adult where fat can be almost exclusively employed (Bennett, 1972). The relationship between age and this apparent change in metabolic capacity is unknown. Also unknown is the possible relationship in the newborn between the rate of heat production and the proportions of carbohydrate and lipid utilised.

1.2.5 Hormones and Heat Production

Hormones and heat production in newborn lambs have been studied in two ways. Either changes in plasma concentrations have been measured during cold stress or the effects of hormone infusion on metabolic rate have been studied.

(a) Thyroid hormones. Plasma thyroxine levels rise in the foetus just before birth (Mellor, Matheson, Small and Wright, 1976) and birth itself and the initial exposure to cold results in increases in the plasma levels of both tri-iodothyronine and thyroxine (Sack, Beaudry, de Lamater, Oh and Fisher, 1976) although most of the lambs included in this study were prematurely delivered by caesarean section and these results must be treated with some caution. There have been no direct estimations of the plasma concentrations of thyroid hormones during cold stress. Infusion of tri-iodothyronine into the newborn

lamb exposed to cold stress did not result in an increase in metabolic rate but sustained treatment over a period of days did result in an increase in summit metabolism on later exposure (Alexander, 1970). Daily treatment with thyroxine was found to reduce the age-related decline in summit metabolism whilst postnatal thyroidectomy increased it (Alexander, 1970).

The thyroid hormones would appear to act as indirect determinants of heat production capacity rather than direct effectors of the heat production processes.

(b) Insulin and glucagon. Plasma insulin levels have been generally found to decrease during cold exposure in parallel with increases in the plasma glucose concentration (Bassett and Alexander, 1971). These changes were most marked in lambs aged two days or more and were reproduced by catecholamine infusion under thermoneutral conditions. No significant changes in metabolic rate during summit metabolism were observed after the infusion of either insulin or glucagon (Alexander, 1970). These observations suggest that in the newborn lamb these hormones are effectors of metabolism and not metabolic rate.

(c) Growth hormone. In lambs aged less than one day, growth hormone levels were found to decrease during cold exposure and to rise during recovery (Bassett and Alexander, 1971). In older lambs very low levels of growth hormone were observed in all states.

(d) Thyrotrophin and corticotrophin. Plasma levels of these two hormones have not been measured during cold stress and neither resulted in any significant change in metabolic rate on infusion during summit metabolism (Alexander, 1970).

(e) Corticosteroids. Plasma corticosteroid levels tend to increase

during cold exposure and decrease during recovery (Bassett and Alexander, 1971). Infusion of hydrocortisone during summit metabolism did not cause any significant change of metabolic rate (Alexander, 1970). Postnatal adrenalectomy resulted in a reduction of summit metabolic rate to 50% of control. The infusion of cortisol elevated this value to 85%. The remaining 15% deficit was restored by the infusion of catecholamines, principally adrenaline (Alexander and Bell, 1982). Adrenal corticosteroid secretion clearly plays a major role in the thermogenic response to cold stress.

(f) Catecholamines. The effects of catecholamine infusion on brown adipose tissue metabolism have already been reviewed and there is no doubt that an increase in sympathetic nervous activity plays an important part in regulating the increase in metabolic rate induced by exposure to cold (Alexander, Mills and Scott, 1968). However, levels of catecholamines during cold stress in the newborn lamb have not been measured.

1.3 INTRODUCTION TO WORK CONDUCTED

Hypothermia is a serious problem in newborn lambs. The studies of heat loss described have provided a good understanding of this aspect of thermoregulation in the newborn lamb but studies of heat production capacity, although extensive, still leave many questions unanswered - the major one being 'why do some lambs produce considerably more heat than others?' One major criticism of the work reviewed is that in many cases the age of the lamb studied was poorly controlled whilst at the same time many aspects of heat production capacity were found to be age-related. Some of the unexplained variations in heat production capacity, especially that found in very young lambs, may have been

related either to age or to age-related factors such as the degree of depletion of body energy reserves. This thesis describes:

1. A controlled study of heat production capacity in newborn lambs aged up to five hours with special emphasis on the sources of lamb to lamb variation. Factors considered include heat production capacity at one, two and five hours of age; substrates for heat production; blood and plasma composition during cold stress; the relationship between heat production capacity and body weight; the effects of hypoxia during birth and in the postnatal period on the subsequent heat production capacity; the effects of feeding.
2. A detailed study of clinical cases of hypothermia with emphasis on the diagnosis of the causes of the condition, and identification of any predisposing factors.
3. The development of new and more effective techniques for treatment.

CHAPTER II

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MATERIALS AND METHODS

2.1 ANIMALS

2.1.1 Breeds

Lambs of two breeds, Dorset Down and Scottish Blackface were used. The Dorset Down ewe breeds earlier than the Scottish Blackface and the use of these two breeds enabled investigations to be conducted from January to May as required.

2.1.2 Source

Lambs of both breeds were obtained from the Moredun Institute flocks. Mating, which was recorded, and management in the first four months of pregnancy was conducted by the Institute farm staff.

2.1.3 Management

Pregnant ewes were brought to the laboratory approximately one month before lambing. The ewes were housed in individual pens fitted with slatted floors. After lambing, the ewes and lambs were maintained in these pens until experimental procedures were completed, after which time housing was dictated by normal shepherding principles. The ewes were fed ad libitum a proprietary complete diet (Ruminant A, Seafield Mill, Roslin, Midlothian). No nutritional restrictions were imposed.

All lambings were observed. Correct presentation was checked at an early stage and the ewes were allowed to lamb without interference if possible. However when progress appeared abnormally slow or absent assistance was provided. Wherever possible the lamb, or lambs, were allowed to remain with the ewe. When fasting was necessary the lamb was placed in an open topped plastic basket placed in the pen which gave the ewe constant access to the lamb whilst preventing sucking.

2.2 MEASUREMENT OF METABOLIC RATE

2.2.1 Principle

Metabolic rate was estimated by indirect calorimetry. Basal metabolic rate was measured whilst the lamb's body was immersed in warm water and summit metabolism was elicited by a progressive cooling of the water until rectal temperature commenced to fall.

2.2.2 Water Immersion and Cooling Procedures

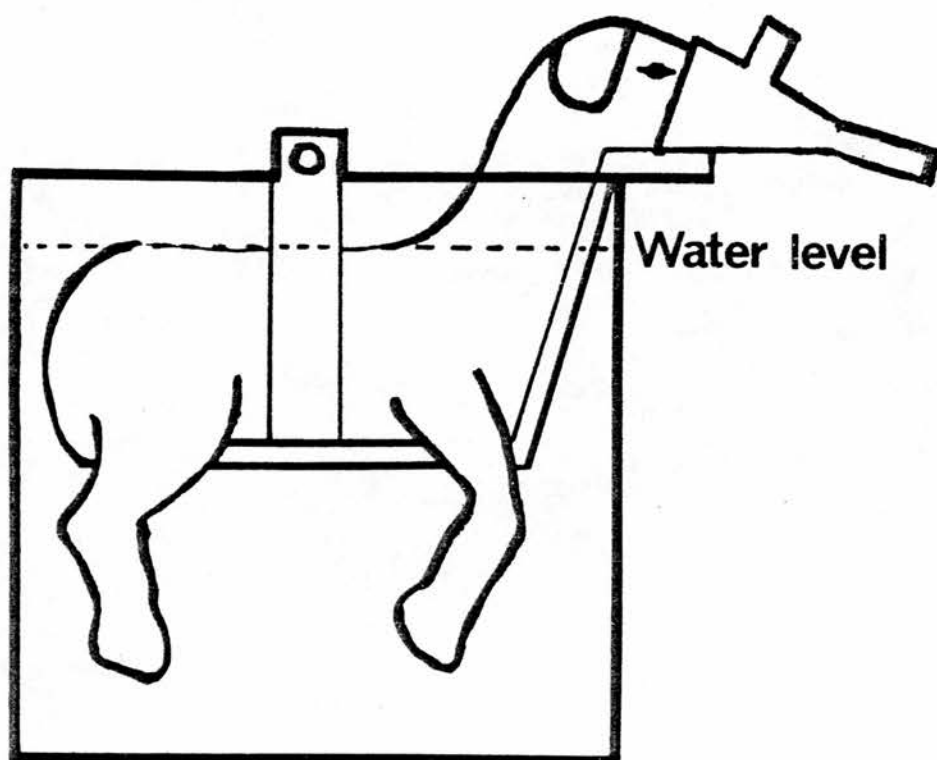
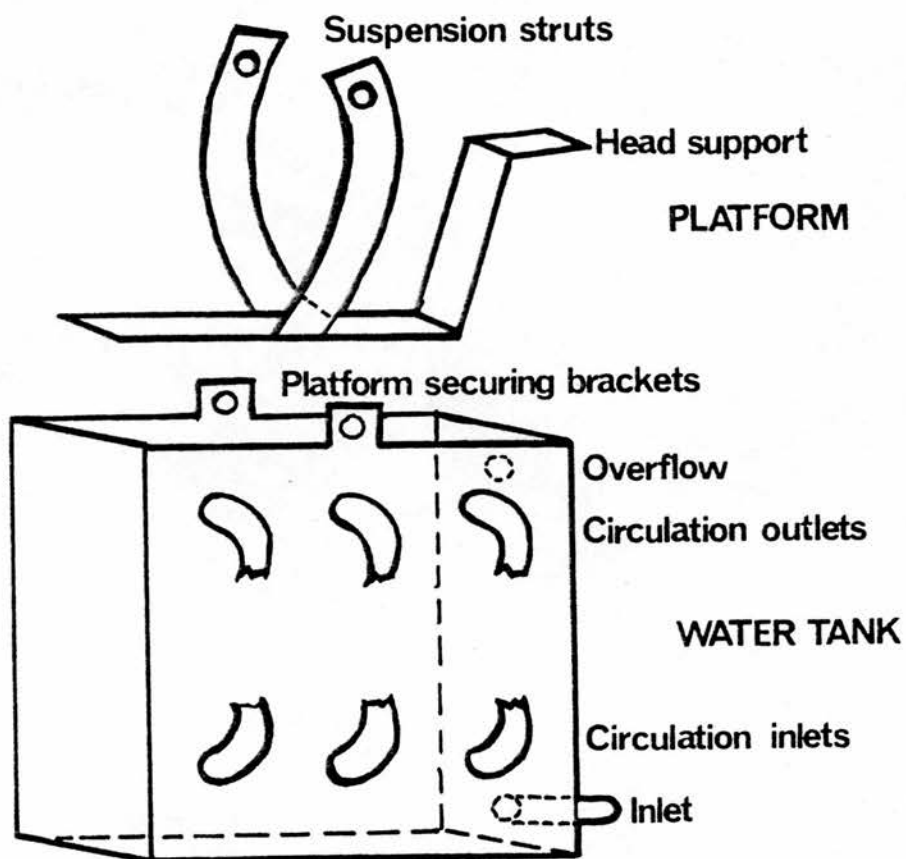
The lamb, supported by a foam lined platform, was placed in a water tank at 38.5°C (Fig. 2.1). The tank was 550 mm deep, 500 mm long and 260 mm wide. Water was introduced just above the tank base at a controlled rate and temperature, and was circulated by means of six pumps. The rate was monitored by an in-line rotameter type flow meter and controlled by a simple valve. The temperature was controlled by a calibrated mixing valve. The water temperature was maintained at 38.5°C, which was found to be thermoneutral for newborn lambs, for twenty minutes after the achievement of a steady metabolic rate (basal metabolic rate). Cold water was then introduced into the tank to cause a progressive fall in the water temperature of 0.5°C per minute. Cooling was continued until rectal temperature had fallen, after an initial rise, to the value observed during basal metabolism (Fig. 2.2). Cooling was then stopped. When rectal temperature had fallen to 37.0°C the lamb was removed from the water and dried. The water temperature at which cooling was stopped varied from lamb to lamb with a range of 14 - 28°C.

2.2.3 Indirect Calorimetry

A foam lined face mask with inlet and outlet ports was fitted to the

Figure 2.1 (a) The water tank and lamb supporting platform. The circulation ports on the far side of the tank have been omitted for the sake of clarity.

Figure 2.1 (b) A lamb in the water tank showing the supporting platform and face mask in place.



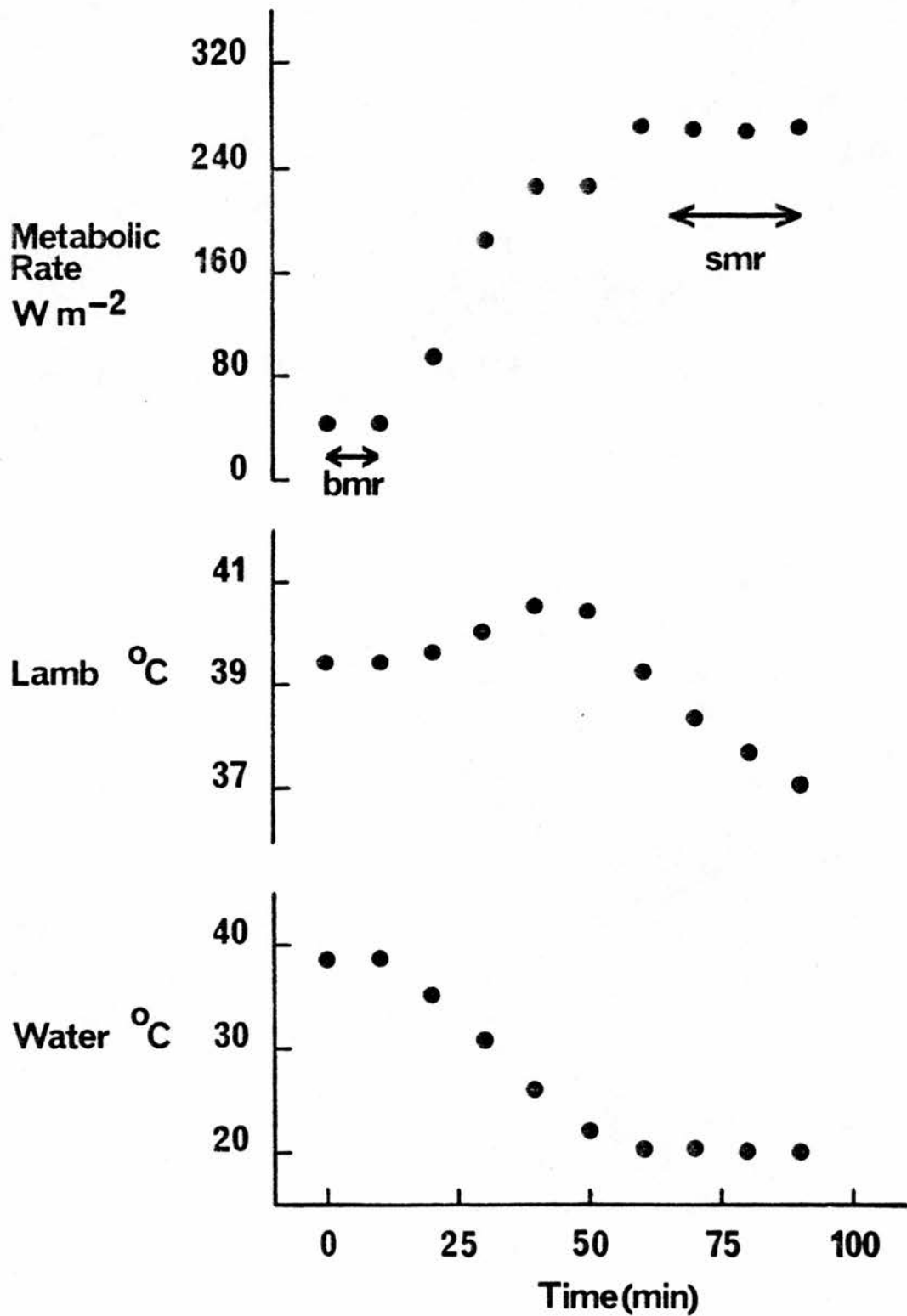


Figure 2.2 Metabolic rate, rectal temperature and water tank temperature during the measurement of basal metabolic rate (bmr) and summit metabolic rate (smr) in a five-hour old Scottish Blackface single lamb of body weight 4.60 kg.

lamb (Figs 2.1 and 2.3). Fresh outside air was drawn through the face mask by means of a pump (Fig. 2.3). The rate of air flow was adjusted to between 6 and 20 litres per minute to maintain the carbon dioxide concentration in mixed expired air at about 1%. The expired air was dried by passage through two vertical columns containing calcium sulphate. The oxygen content of the mixed dried expired air was estimated with a paramagnetic analyser (Type OA137, Taylor - Servomex) and the carbon dioxide content with an infra-red analyser (Model 2, Sir Howard Grubb Parsons)(Fig. 2.3). Mass dry air-flow was estimated with a pressure drop transducer (Model EA11 - 50KX, Teledyne Hastings-Raydist). The gas analysers were calibrated before, during and after estimations using dry fresh air (assumed to contain 20.95% oxygen and no carbon dioxide) and previously calibrated gas mixtures. The flow transducer was calibrated by coupling to a wet gas meter (Type DM3E, Alexander Wright). The system was regularly tested for leaks. Joints under positive pressure were tested by the application of soapy water. Joints under negative pressure were surrounded with an atmosphere of nitrogen and leaks detected by changes in the exhausted air oxygen concentration.

The expired air oxygen and carbon dioxide contents together with mass air flow were continuously recorded on a multiple channel flat-bed chart recorder. Values for each of these three measurements at 30 second intervals were translated onto punched tape together with appropriate calibration points. Metabolic rate was then calculated according to Brouwer (1965) with the aid of a computer. Metabolic rate was expressed per unit body weight and per unit body surface area, the latter derived according to Peirce (1934). The average metabolic rate during the last ten minutes of thermoneutral measurement was taken as

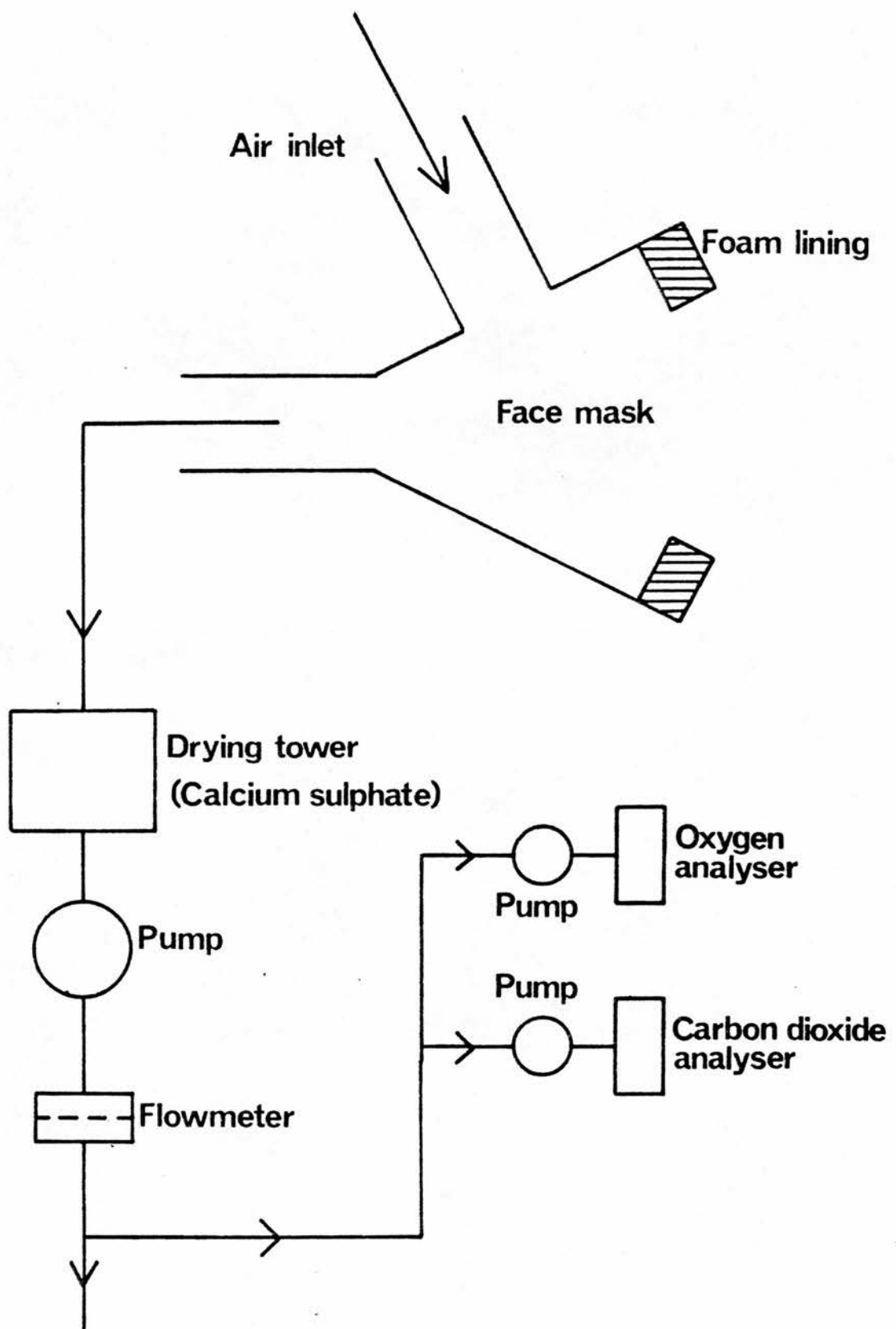


Figure 2.3 A cross-section of the face mask and a scheme of the open circuit indirect calorimetry system.

an estimation of basal metabolic rate. Summit metabolism was calculated as the average metabolic rate for a ten minute period which commenced when rectal temperature had fallen to 39.0°C. Values obtained when rectal temperature had fallen below 37.0°C were not included, since below this temperature metabolic rate has been found to be body temperature dependent (Alexander, 1962b). In a few lambs this resulted in the period of measurement being less than 10 minutes. The minimum period was 7 minutes.

2.3 MEASUREMENT OF TEMPERATURE

Rectal temperature was measured using a thermistor probe, the sensor tip of which was inserted 6 cm into the rectum. Water temperature was measured using a thermistor bead situated in one of the circulation outlet ports of the tank. Both these temperature sensors were calibrated in water against a '0.05°C' mercury-in-glass thermometer and values were continuously recorded on the chart recorder.

2.4 BLOOD SAMPLING

2.4.1 Umbilical Vein

Blood samples obtained during experiments involving water immersion were taken from the posterior vena cava via a catheter inserted into one of the umbilical veins immediately after birth. The technique of Mellor and Pearson (1977) was employed except that the catheter was passed via an incision in the wall of the vein and not into the open end. Samples were taken into either 2 or 5 ml syringes heparinised to give a final heparin concentration of 0.08 mg/ml. Catheter patency was preserved with a heparin/saline solution containing 3 mg heparin and 9 mg sodium chloride per ml. On the completion of an experiment the

catheter was removed whilst simultaneously injecting into it 5 ml of a solution containing 60 mg/ml sodium benzyl penicillin. In addition penicillin was administered parenterally for the next five days (Propen, Glaxo). This antibiotic regime considerably reduced the incidence of post-experimental infection which can follow the use of umbilical vein catheters in lambs.

2.4.2 Jugular Vein

All other blood samples were taken by needle puncture of a jugular vein employing a one inch 20 gauge needle and a heparinised syringe as described above. No problem of infection followed the use of this technique.

2.5 SAMPLE STORAGE

2.5.1 Blood and Plasma

After sampling the syringe was capped, mixed and placed in iced water. Blood for pH and gas tension analysis was stored for up to two hours. Blood for haematological examinations was stored at +4°C for up to 24 hours. After a minimum of 30 minutes in iced water, plasma was separated from blood by refrigerated centrifugation, and was then stored at -40°C until analysis.

2.5.2 Storage of Samples for Lactate and Pyruvate Estimations

There are suggestions in the literature (Hohorst, 1963) that lactate and pyruvate are unstable in stored samples and it is generally advocated that analysis should be performed immediately after sampling. The nature of the work described here made this extremely inconvenient and storage for several months before analysis was highly desirable. The effects of storage on the stability of both lactate and pyruvate

under different conditions were thus investigated as described below.

A 200 ml sample of heparinised sheep blood was chilled in iced water. A plan of subsequent procedures, which were conducted at $+4^{\circ}\text{C}$, is shown in Figure 2.4. Deproteinisation was conducted by mixing the sample with two volumes of 0.8 M perchloric acid. After centrifugation the three fractions (blood acid supernatant, plasma acid supernatant and plasma) were each dispensed into 10 ml vials, 5 ml of sample being placed in each vial. One vial of each fraction was then immediately assayed for lactate and pyruvate (see 2.6.1 for methods). Further vials of each of the three fractions were then stored as shown in Figure 2.4. The maximum deviation from the stated storage temperatures was 2°C . Further estimations were performed after 15, 35, 59 and 136 days of storage. A fresh vial of sample was used for each estimation which was replicated five times.

No significant changes in the concentrations of lactate were observed after storage for 136 days. From the estimations made on samples stored for 0, 15, 35, 59 and 136 days ($n=5$) the mean values ($\pm\text{SD}$) obtained were 4.0 ± 0.02 mM in blood acid supernatant at -39°C , 4.9 ± 0.04 mM in plasma acid supernatant at -39°C , 4.9 ± 0.17 mM in plasma at -39°C , 5.0 ± 0.12 mM in plasma at -23°C , 4.9 ± 0.12 mM in plasma at -7°C and 4.9 ± 0.10 mM in plasma at $+2^{\circ}\text{C}$.

Pyruvate was stable for 136 days only in plasma acid supernatant or plasma stored at -39°C (Figs. 2.5 and 2.6). In the other stored fractions a time-related loss of pyruvate was observed. In the case of plasma, the rate of loss was time and temperature-dependent (Fig. 2.6). A possible explanation for the loss of pyruvate from plasma samples stored at temperatures above -39°C could have been conversion to lactate, an exergonic reaction catalysed by lactate

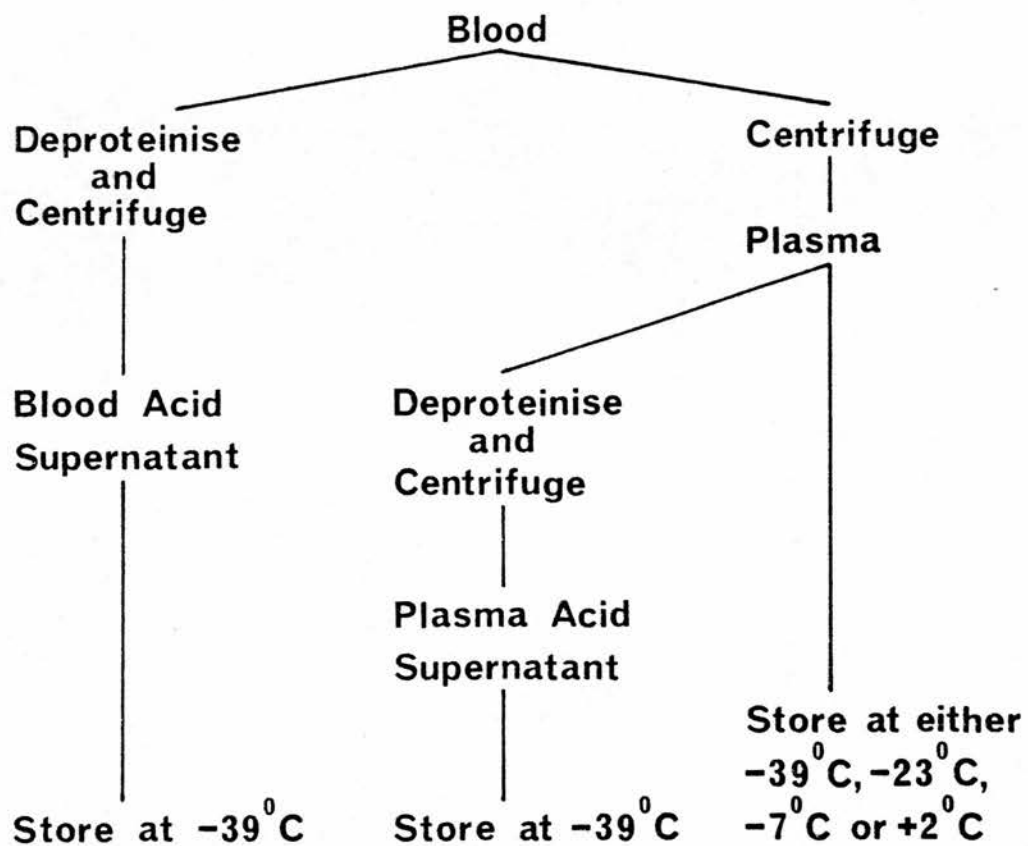


Figure 2.4 The effects of storage on the stability of lactate and pyruvate in blood and plasma; treatments and storage temperatures studied.

Figure 2.5 The stability of pyruvate in plasma acid supernatant (PAS) and blood acid supernatant (BAS) stored at - 39°C for 135 days. The results are expressed as % of the day 0 values (PAS; 0.22 mM, BAS; 0.17 mM).

Figure 2.6 The effect of storage temperature on the stability of pyruvate in plasma for 135 days. The results are expressed as % of the day 0 value (0.22 mM).

Fig. 2.5

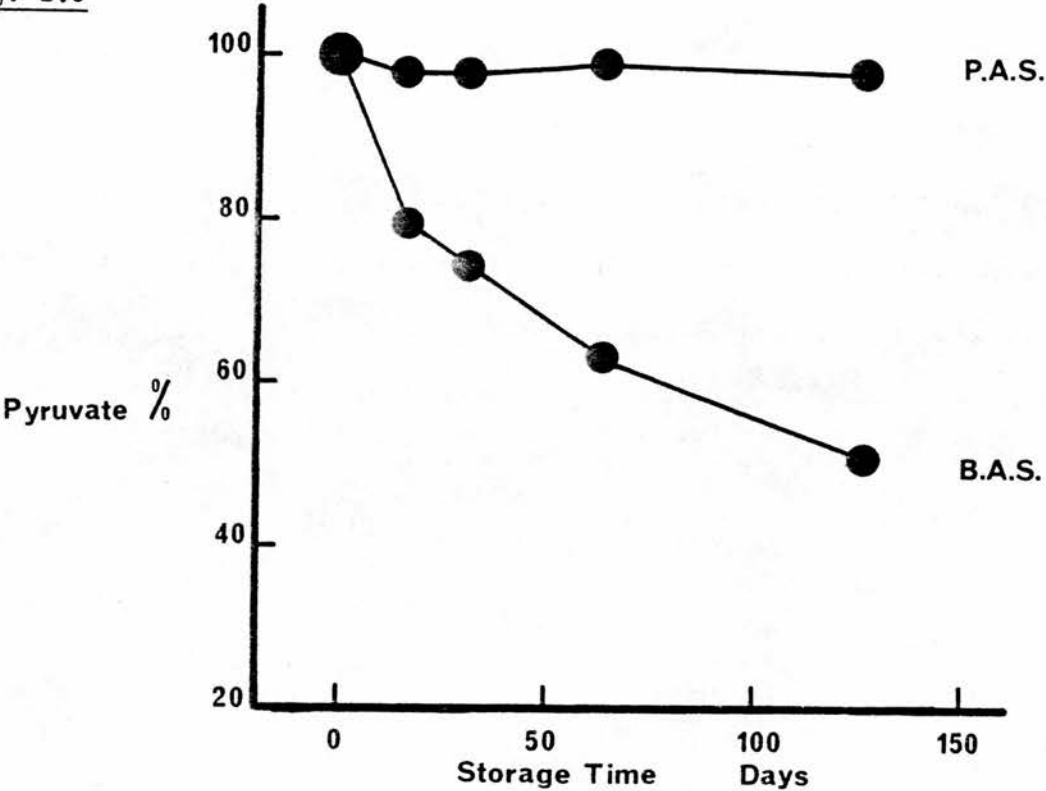
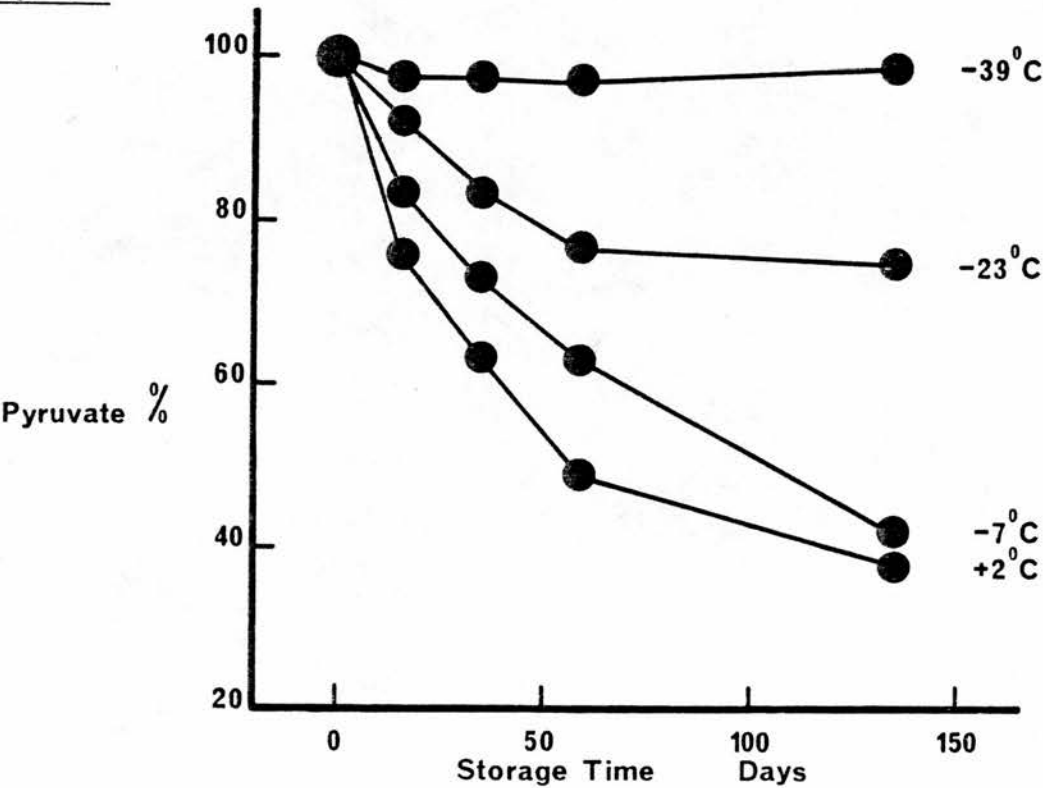


Fig. 2.6



dehydrogenase. The proportionately small increase in the concentration of lactate which would have resulted from this conversion would have been undetectable. Such a conversion would seem an unlikely explanation for the marked loss of pyruvate in blood acid supernatant stored at -39°C . However this was the only stored fraction which contained products of red blood cell destruction and it could be that these products in some way accelerated the loss.

These results demonstrated that only plasma or plasma acid supernatant maintained at -39°C was satisfactory for future pyruvate estimations. Plasma was required for other estimations and thus storage of this sample at approximately -40°C was adopted.

2.5.3 Milk

Milk samples were stored at -40°C until analysis.

2.6 ANALYTICAL METHODS

2.6.1 Blood

(a) Haemoglobin. The blood haemoglobin concentration was estimated by the cyanomethaemoglobin method (Kampen and Zijlstra, 1961).

(b) Red blood cells. Red blood cell counts were performed using a Coulter counter.

(c) Packed cell volume. Packed cell volume was estimated using a micro-haematocrit centrifuge.

(d) Blood pH. Blood pH was estimated using a micro-pH electrode maintained at 39.0°C (Instrumentation Laboratory (UK) Ltd). The electrode was calibrated with buffers of pH values 6.840 and 7.384. The final calibration before sample estimation was conducted with the

buffer of pH value closest to that expected in the sample.

(e) Blood gas tensions. Blood partial pressures of carbon dioxide (PCO_2) and oxygen (PO_2) were estimated using the appropriate electrodes of the system noted above. Both electrodes were calibrated using two water-saturated gas mixtures containing known amounts of carbon dioxide and oxygen. Wherever possible the final calibrations were performed using the gas mixture of carbon dioxide and oxygen tensions nearest to those expected in the sample.

(f) Base excess. Base excess was calculated from the alignment nomogram of Siggaard-Anderson (1963) using the measured pH, pCO_2 and blood haemoglobin concentration. Base excess was used to monitor changes in metabolic acid-base status and no correction was made for the use of ovine blood.

(g) Lactate. Lactate concentration was estimated according to Gutmann and Wahlefeld (1974). In this method the concentration of lactate was estimated by measuring the production of reduced nicotinamide adenine dinucleotide (NADH) when a perchloric acid supernatant of whole blood was incubated with a glycine buffer containing hydrazine, oxidised nicotinamide adenine dinucleotide (NAD) and lactate dehydrogenase. The hydrazine was included in the incubation medium to ensure completion of the reaction by complexing with the pyruvate produced. The increase in the concentration of NADH was estimated as the change in absorbance at 340 nm and the original lactate concentration was calculated directly from this. The result so obtained was checked against an aqueous lactate standard. 'Precinorm S' (Boehringer Corporation, London, Ltd) was used as a quality control.

(h) Pyruvate. The blood concentration of pyruvate was estimated according to Czok and Lamprecht (1974). This method is basically the reverse of the lactate method described above. A perchlorate supernatant of whole blood was neutralised with phosphate buffer. After centrifugation the resulting supernatant was incubated in a solution containing NADH and lactate dehydrogenase. The sample pyruvate concentration was calculated directly from the decrease in the absorbance of NADH at 340 nm. The results so obtained were checked against aqueous standards.

(j) Excess lactate. Excess lactate was calculated according to Huckabee (1958). This value was employed as an index of the degree of tissue hypoxia to which a lamb was exposed during a period of observation.

2.6.2 Plasma

(a) Sodium and potassium ions. The concentrations of sodium and potassium ions were estimated using a flame photometer (IL343, Instrumentation Laboratory (UK) Ltd) which employed lithium nitrate as an internal standard. Aqueous external standards were used.

(b) Lactate and pyruvate. These metabolites were estimated in plasma as described for blood (2.6.1 g and h).

(c) Glucose. Glucose was estimated according to Trinder (1969). The oxidation of glucose by glucose oxidase produces hydrogen peroxide which in turn is reduced by peroxidase with the oxidation of 4-amino phenazone in the presence of phenol to produce a purple colour. The degree of colour development at 515 nm is directly proportional to the initial concentration of glucose. Aqueous standards were used and a commercial standard serum (Versatol, W.R. Warner and Co. Ltd)

was used as a quality control.

During field work when laboratory facilities were not available, it was often desirable to obtain an estimate of the plasma glucose concentration. For this purpose a modification of the method of Gutteridge and Wright (1968) was employed which did not involve a protein precipitation step. This method employed the same principle described above utilizing an extract of guaiacum gum as colour agent. The method, which was developed by Dr J. Proffitt of the Department of Clinical Chemistry at the Royal Infirmary of Edinburgh is described below.

Reagents

Guaiacum extract: 12g guaiacum gum powder (Hughes and Hughes) was mixed with 200 ml ethanol for 2 hours and was then filtered.

Working reagent: An acetate buffer of pH value 6.2 was prepared by dissolving 370 g sodium acetate in 5 litres distilled water to which was added 4.2 ml glacial acetic acid. To this solution were added: 50 mg peroxidase (peroxidase RZ 0.6, Hughes and Hughes), 80 ml Triton x 100 (BDH), 30 ml glucose oxidase solution (Fermocozyme 653AM, Hughes and Hughes), 25 ml guaiacum gum extract and 5 g magnesium sulphate. The reagent was then made up to 6 litres with distilled water and stored at +4°C.

Standards

A stock standard of 100 mM glucose solution was prepared by dissolving 1.802 g D glucose in 100 ml distilled water. Working standards of 0.5, 1.0, 2.0, 5.0 and 10 mM were prepared by appropriate dilutions with distilled water.

Method

1. 5 ml of reagent were dispensed into the required number of test tubes (blank + 5 standards + samples) and the tubes were allowed to

equilibrate at room temperature for one hour.

2. 25 μ l of plasma or standard were carefully introduced into the test tube being allowed to run gently down the inside wall.
3. The tube contents were gently mixed.
4. After 15 minutes the degree of blue coloration in a sample was compared visually with that in the standards and an assessment of the plasma glucose concentration made. If the concentration of glucose in the sample did not lie in the range of 0.5 to 10.0 mM the test was re-run using either double or half the volume of plasma as appropriate.

The technique provided a clinically useful estimation of the plasma concentration of glucose. More accurate estimations were later made in the laboratory employing the technique previously described.

(d) Free fatty acids. The plasma concentration of free fatty acids was estimated according to Lauwerys (1969). In this method a Dole (1956) extract of plasma is shaken with a solution containing chloroform and copper nitrate. This step yields chloroform-soluble copper/free fatty acid salts. The addition of a copper complexing agent, sodium diethyl dithiocarbamate, to the chloroform layer yields a yellow colour, the absorbance of which at 428nm is proportional to the concentration of free fatty acids in the original sample. The actual concentration was derived by reference to palmitic acid standards dissolved in heptane which were run in parallel with the samples. A commercial quality control serum was used, Precinorm S (Boehringer Corporation, London, Ltd).

(e) Glycerol. The plasma glycerol concentration was estimated according to the method of Eggstein and Kuhlmann (1974) using a commercial kit (Test Combination No. 15989, Boehringer Corporation,

London, Ltd). In this method glycerol is first phosphorylated with the production of adenosine diphosphate (ADP). The ADP is then in turn phosphorylated to adenosine triphosphate (ATP) with the conversion of phospho-enol pyruvate to pyruvate. Finally the pyruvate is reduced to lactate by NADH. The decrease in the absorbance of NADH at 340 nm is proportional to the amount of glycerol in the original sample.

Precinorm S (Boehringer Corporation, London, Ltd) was used as a quality control.

(f) Triglycerides. The plasma concentration of triglycerides was estimated as glycerol following saponification of the sample with alcoholic potassium hydroxide. The free glycerol value was subtracted from the result so obtained to achieve the triglyceride glycerol concentration and thence the triglyceride concentration itself. Reagent grade olive oil dissolved in an ether-ethanol mixture was used as a quality control.

(g) β -hydroxybutyrate. β -hydroxybutyrate was estimated according to the method described by Chandrasekaran, Glover and Lord (1972). In this method β -hydroxybutyrate is oxidised to aceto-acetate by β -hydroxybutyrate dehydrogenase with the conversion of NAD to NADH. Hydrazine is incorporated in the incubation mixture to ensure completion of the reaction. The concentration of β -hydroxybutyrate in the sample was calculated directly from the increase in the concentration of NADH estimated by change in absorbance at 340 nm. The result so obtained was checked by reference to aqueous standards.

(h) Total proteins. The total protein concentration was estimated by the standard Biuret method. In alkaline solution protein forms a coloured complex with copper ions. The protein concentration is estimated by measuring absorbancy at 550 nm. Albumin in aqueous solution

was employed as a standard and Precinorm S (Boehringer Corporation, London, Ltd) as a quality control.

(j) Urea. The plasma concentration of urea was estimated according to the method of Marsh, Fingerhut and Miller (1965). In this method urea is condensed with diacetyl derived from the breakdown of diacetyl monoxime to yield a red colour. The intensity of the colour produced, measured by absorbance at 518 nm, is proportional to the concentration of urea in the sample. Aqueous solutions of urea were employed as standards and Precinorm S (Boehringer Corporation, London, Ltd) was used as a quality control.

(k) Immunoglobulin G (IgG). The plasma concentration of IgG was estimated by the method of Mancini, Carbonara and Heremans (1965) as modified by Fahey and McKelvie (1965). In this method a 1 in 20 dilution of plasma is placed in a small well cut in an agar gel plate into which anti-IgG antiserum has been evenly incorporated. A concentric ring of antigen/antibody precipitate forms around the antigen well and the diameter of this ring is proportional to the quantity of IgG present in the sample. By reference to standards the IgG concentration in the original plasma is calculated. The antiserum was raised against sheep IgG₁ and IgG₂ in pigs. Sheep IgG (Sigma) dissolved in an IgG - free lamb plasma was employed as the standard.

(l) Corticosteroids. The plasma concentration of corticosteroids was estimated by a modification of the competitive protein binding method of Bassett and Hinks (1969). A plasma sample was deproteinised with ethanol and tritiated cortisol added to an aliquot of the supernatant. After freeze-drying, a solution of corticosteroid-binding globulin, prepared from dog plasma, was added. After incubation and

cooling in iced water to bring the reaction to equilibrium, the free cortisol was separated by the addition of a dextran-coated charcoal solution. The concentration of tritiated cortisol in the supernatant was estimated by scintillation counting. If a sample contained little steroid, a high proportion of the tritiated cortisol was bound to the corticosteroid-binding globulin with a resulting high level of activity in the counted sample. If, on the other hand, the sample contained a high level of endogenous steroid, comparatively little tritiated cortisol was bound to the cortisol-binding globulin resulting in a low level of activity in the counted sample. Standards were prepared by adding different dilutions (5-70 $\mu\text{g/ml}$) of cortisol to corticosteroid-free ovine plasma. Two batches of standards were run in each assay to estimate within assay drift. A pooled lamb plasma was included in each assay as quality control. The mean ($\pm\text{SEM}$) concentration was 75.3 ± 2.46 nM for five assays and the inter-assay coefficient of variation was 7.3%. The plasma corticosteroid concentration was expressed as cortisol (nM).

(m) Insulin. The plasma concentration of insulin was estimated by the radio-immunoassay method described by Hales and Randle (1963). Insulin labelled with $^{131}\text{I}_2$ is added to the sample and then a binding agent is added. This agent contained a preprecipitated double antibody comprising guinea-pig anti-insulin serum which binds insulin and rabbit anti guinea-pig gamma globulin serum which binds and precipitates the guinea-pig gamma globulin. After centrifugation the activity of iodine in the precipitate is counted. A high concentration of insulin in the sample results in a low level of activity in the precipitate whereas a low concentration of insulin results in a high level of activity. Results were calculated by reference to

standards (human insulin standard, Wellcome R P 13 reconstituted in phosphate buffered bovine albumin solution). A pooled lamb plasma was included in each assay and the mean (\pm SEM) was 83.6 ± 2.73 pM for seven assays with an inter-assay coefficient of variation of 8.6%.

(n) Catecholamines. Adrenaline, noradrenaline and dopamine in plasma were estimated according to the method of Da Prada and Zurcher (1976) with several modifications. The principles employed in this method are as follows. The three catechols are converted to their ^3H -methylated derivatives by the enzyme catechol-O-methyl transferase using ^3H -S-adenosyl methionine as methyl group donor. The tritiated methyl derivatives are then separated from excess methyl donor by extraction into diethyl ether and back extraction into 0.1 M hydrochloric acid. Sample volume is reduced by freeze drying and then a reconstituted sample is spotted onto a silica gel plate (Linear - K preabsorbent TLC plates, Whatman). After development with a chloroform-methanol-70% ethylamine mixture in the proportions 16:3:2 the bands containing methoxytyramine (methylated dopamine) normetanephrine (methylated noradrenaline) and metanephrine (methylated adrenaline) are located under ultra violet light and scraped into separate containers. Methoxytyramine is eluted from the silica gel with borate buffer complexed with tetraphenol borate and is then extracted into a toluene based liquid scintillation counting solution for estimation of the ^3H content. Normetanephrine and metanephrine are eluted with 0.05 M ammonium hydroxide solution and then oxidised to vanillin with sodium periodate. The solutions are then acidified with acetic acid and extracted into a toluene based liquid scintillation counting solution for estimation of the ^3H content. Internal standards were employed, each sample being run with and without added catecholamines.

A pooled lamb plasma was included in each assay. The mean (\pm SEM) concentration of adrenaline was 8.5 ± 0.40 nM ($n = 6$), noradrenaline 13.6 ± 0.56 nM ($n = 8$) and dopamine 5.0 ± 0.33 nM ($n = 5$) with coefficients of variation of 11.4%, 11.7% and 14.6% respectively. The within assay variation was assessed by five replicate analyses of three plasmas. The mean (\pm SEM) values were:

Plasma 1

Adrenaline:	5.3 ± 0.16 nM	Coefficient of variation	6.9%
Noradrenaline:	14.8 ± 0.44 nM	" " "	6.6%
Dopamine:	3.9 ± 0.11 nM	" " "	6.5%

Plasma 2

Adrenaline:	11.6 ± 0.32 nM	Coefficient of variation	6.1%
Noradrenaline:	31.9 ± 0.87 nM	" " "	6.1%
Dopamine:	8.4 ± 0.34 nM	" " "	9.0%

Plasma 3

Adrenaline:	59.1 ± 1.32 nM	Coefficient of variation	5.0%
Noradrenaline:	162 ± 2.3 nM	" " "	3.1%
Dopamine:	38.0 ± 0.61 nM	" " "	3.6%

Least variation was observed in the plasma with the highest levels of catecholamines.

The modifications to the original method were:

1. Methylation of catechols. The maximum degree of methylation was obtained with the following changes in reagent concentrations:
 - (a) Catechol-O-methyl transferase concentration was doubled.
 - (b) The concentration of 1-2 di (2-amino-ethoxy) ethane-N, N, N', N' tetra acetic acid incorporated to bind calcium ions which inhibit methylation was increased by a factor of 1.5.

2. Elution of methyl derivatives from the silica gel.

(a) Dopamine. The recommended procedure of elution with hydrochloric acid and addition to an ethanol-containing liquid scintillation counting solution resulted in a very high degree of chemiluminescence. Elution with borate buffer as already described overcame this problem.

(b) Adrenaline and noradrenaline. 0.5 ml of 0.05 M ammonium hydroxide solution was used for elution instead of 1 ml of 2 M solution. This modification was just as effective as the original technique but avoided the use of concentrated alkali, an unpleasant and dangerous procedure.

(c) Thyroxine. The plasma concentration of thyroxine was estimated according to the radio-immunoassay technique of Seth, Toft and Irvine (1976). In this technique the sample is incubated with anti-ovine thyroxine antibody bound to micro-crystalline cellulose, and labelled thyroxine ($3, 5 \text{ }^{125}\text{I}_2 - \text{L} - \text{thyroxine}$). After incubation the solution is centrifuged to separate bound from free thyroxine and the bound labelled thyroxine estimated by counting. A high concentration of thyroxine in the original sample results in a low quantity of labelled thyroxine being bound and vice versa. Standards were prepared by adding known concentrations of thyroxine to thyroxine-free bovine plasma which was obtained by charcoal extraction. A pooled lamb plasma was included in each assay. The mean (\pm SEM) concentration was $155 \pm 4.1 \text{ nM}$ for four assays and the inter-assay coefficient of variation was 5.3%.

(d) Tri-iodothyronine (T_3). The plasma T_3 concentration was estimated in a manner similar to that described for thyroxine (Seth, Toft and Irvine, 1976) using anti- T_3 antibody, $^{125}\text{I}_2$ labelled T_3 and T_3 standards.

A pooled lamb plasma was included in each assay. The mean (\pm SEM) concentration was 5.21 ± 0.164 nM for four assays and the inter-assay coefficient of variation was 6.3%.

2.6.3 Milk

The lipid content of milk was estimated as triglycerides. The method outlined in 2.6.2 (f) was employed using a 1:100 aqueous dilution of milk in place of plasma. The lactose content of milk was estimated as total reducing substances according to the method described by Asatoor and King (1954). In this method cupric ions in alkaline solution are reduced to cuprous ions which then reduce phosphomolybdic acid with the formation of a blue colour. The lactose content of the sample is derived from reference to absorbance at 600 nm. Aqueous standards were employed. The total solid content of the colostrum was measured by freeze drying and an estimation of protein content was made by subtracting the weights of triglyceride and lactose from the dry weight.

2.7 ANALYSIS AND PRESENTATION OF RESULTS

The results were analysed using the student's t tests (for the comparison of means from independent and non-independent samples), covariance analysis (Snedecor and Cochran, 1967) and regression analysis. Mean values are presented with the standard error of the mean (SEM) unless otherwise indicated. The results of regression analysis are shown as coefficients of correlation (r). The number of observations used to compute these values (n) is given in brackets. The statistical significance of findings are indicated as probabilities. The notations *, **, and *** are used to indicate probabilities of $P < 0.05$, < 0.01 and < 0.001 respectively. The distributions of the plasma levels of

insulin, adrenaline, noradrenaline and dopamine were 'normalised' before statistical analysis by logarithmic transformation.

CHAPTER III

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HEAT PRODUCTION

3.1 BASAL METABOLISM

3.1.1 Introduction

Basal metabolic rate has no direct association with the capacity of a lamb to produce heat under cold conditions. However, it was considered desirable to estimate basal metabolic rate for three reasons:

1. To demonstrate that the water immersion and indirect calorimetry procedures themselves were not stressful and that any increase in heat production recorded during cold stress was a response to the cold stress alone and not some combination of the cold stress and the experimental conditions.
2. To establish basal values for plasma and blood composition in order to be able to interpret values recorded during summit metabolism.
3. To establish the magnitude of the increase in heat production which could be elicited by cold exposure.

Basal metabolic rate was measured during immersion in water at 38.5°C. The major reason for the choice of this technique was the later use of cold water for the elicitation of summit metabolism. However, it did seem likely that this might be an effective technique since the environment resembled in a limited way the warm fluid environment which the lamb had recently occupied.

3.1.2 Methods

Fasting basal metabolic rate was elicited for a ten minute period in 24 Scottish Blackface lambs aged 1½ hours (mean weight 4.08 ± 0.166 kg, comprising five male singles, six female singles, four male twins and nine female twins) and in 30 Scottish Blackface lambs aged 4½ hours

(mean weight 4.15 ± 0.151 kg, comprising seven male singles, four female singles, ten male twins and nine female twins). The estimations were continued for a further 70 minutes in ten of the lambs (five aged $1\frac{1}{2}$ hours and five aged $4\frac{1}{2}$ hours) in order to investigate the effect of prolonged thermoneutral water immersion. Blood samples were taken from some of the lambs at the end of the 10 minute measurement period. Five of the lambs (all aged $1\frac{1}{2}$ hours) subjected to prolonged thermoneutral immersion were further sampled at 20 minute intervals.

3.1.3 Results

(a) Behaviour. On initial immersion in the warm water at 38.5°C the lambs were restless but within 5-10 minutes they settled into a sleep-like state. Their eyes were shut and the lambs were apparently totally relaxed. On a few occasions apnoea was observed for periods of up to 20 seconds.

(b) Metabolic rate. On initial immersion the metabolic rate was two to three times higher than the lowest rate that was subsequently observed. Over a period of 10-25 minutes this rate progressively decreased until a steady nadir was observed which was taken as basal metabolic rate. Mean basal metabolic rate in the $1\frac{1}{2}$ hour old lambs was 3.70 ± 0.142 W/kg (53.5 ± 1.55 W/m²)($n = 24$) and in the $4\frac{1}{2}$ hour old lambs 3.22 ± 0.143 W/kg (46.1 ± 2.26 W/m²)($n = 30$). The rate in the $4\frac{1}{2}$ hour old lambs was significantly less than that in the younger lambs ($P < 0.05$ for both W/kg and W/m²). In both groups of lambs, basal metabolic rate was positively correlated with body weight (lambs aged $1\frac{1}{2}$ hours $r = 0.663$, $p < 0.001$, $n = 24$, and lambs aged $4\frac{1}{2}$ hours $r = 0.588$, $p < 0.001$, $n = 30$).

There were no relationships between basal metabolic rate per unit

weight and rectal temperature (mean $39.4 \pm 0.05^\circ\text{C}$, $n = 54$), sex, litter size or gestational age.

(c) Respiratory quotient. Mean respiratory quotient in the $1\frac{1}{2}$ hour

old lambs was 0.88 ± 0.015 ($n = 24$) and in the lambs aged $4\frac{1}{2}$ hours,

These values were not significantly different.

Relationships between respiratory quotient and any other

Mean values are shown in Table 3.1. A comparison

lambs showed significantly higher values in the

pH and haemoglobin concentration and the plasma

rate, β -hydroxybutyrate and tri-iodothyronine.

Values were observed for PvCO_2 and the plasma concentration.

and thermoneutral immersion. The sleep-like state

measurement of basal metabolic rate was maintained

period in the ten lambs which were kept in the

for 70 minutes. Occasionally short periods of

'wakefulness' were observed which were associated with increased physical activity and an increase in metabolic rate of up to twice the basal value.

However, these periods only lasted a few minutes and the sleep-like state

was then resumed. When the total period was considered no significant

changes in metabolic rate, rectal temperature or respiratory quotient

were observed. The only significant change in blood composition was a

progressive lowering of the plasma glycerol level from 229 ± 28.7 to

$82 \pm 21.9 \mu\text{M}$ ($P < 0.01$, $n = 5$).

3.1.4 Discussion

The mean basal metabolic rate observed in the $4\frac{1}{2}$ hour old lambs in warm

immunity metabolism
cross - what happens
 O_2 consumption
measurement & made
clear 37°C ,
need of cooling
intended.

Table 3.1 Blood and plasma composition in fasted Scottish Blackface lambs aged either 1½ or 4½ hours during the estimation of basal metabolism, mean ± SEM (n). A significant age related difference is indicated.

	<u>1½ hours old</u>		<u>4½ hours old</u>
<u>Blood</u>			
Red cell count 10 ⁶ mm ⁻³			9.04 ± 0.302 (11)
Packed cell volume %	40 ± 2.6 (12)		39 ± 1.3 (18)
Haemoglobin gl ⁻¹	105 ± 9.9 (12)	*	132 ± 4.6 (18)
pH	7.33 ± 0.024 (12)	*	7.39 ± 0.012 (18)
PvCO ₂ kPa	7.5 ± 0.24 (13)	*	6.7 ± 0.19 (17)
Base excess mEq l ⁻¹	3 ± 2.1 (12)		5 ± 1.2 (18)
PvO ₂ kPa	5.3 ± 0.27 (13)		5.7 ± 0.25 (17)
Lactate mM	3.85 ± 0.911 (13)		3.50 ± 0.568 (7)
Pyruvate μM	160 ± 15 (13)	**	233 ± 24 (7)
<u>Plasma</u>			
Glucose mM	2.09 ± 0.395 (13)		3.00 ± 0.400 (18)
Free fatty acids mM	0.39 ± 0.040 (13)		0.45 ± 0.034 (18)
Triglycerides μM	130 ± 21.8 (13)	*	78 ± 6.7 (18)
Glycerol μM	148 ± 16.5 (13)		104 ± 37.2 (18)
β-hydroxybutyrate μM	44 ± 4.3 (11)	*	63 ± 5.7 (18)
Total Protein gl ⁻¹	41.4 ± 2.21 (13)		39.1 ± 1.13 (7)
Urea mM	-		5.36 ± 0.207 (11)
Adrenaline nM	-		0.91 ± 0.171 (9)
Noradrenaline nM	-		3.82 ± 0.648 (10)
Dopamine nM	-		0.87 ± 0.088 (6)
Thyroxine nM	134 ± 13.9 (13)		145 ± 9.8 (7)
Tri-iodothyronine nM	4.13 ± 0.347 (13)	**	6.31 ± 0.412 (7)
Insulin pM	140 ± 39.1 (13)		80 ± 15.1 (18)
Cortisol nM	48 ± 6.1 (13)		69 ± 10.8 (7)

water of 3.22 ± 0.143 W/kg is almost the same as that estimated in warm air by Andrews, Mercer, Ryan and Szekely (1973) of 3.21 ± 0.075 W/kg ($n = 29$) in cross-bred lambs aged 1-6 hours. The mean basal metabolic rate in our younger lambs was significantly higher than in our older lambs and than in those of Andrews *et al.* (1973), ($P < 0.01$). The higher basal metabolic rate in the younger lambs may be a reflection of the substantial increase in metabolic rate that occurs immediately after birth.

Blood composition during basal metabolism has been described elsewhere (Alexander and Mills, 1968; Alexander, Mills and Scott, 1968; Bassett and Alexander, 1971; Alexander, Bell and Hales, 1972). In each case comparison with our values is confounded by the effects of age, breed, feeding, treatment after birth and the technique used to estimate basal metabolic rate. However, when allowance is made for these effects, there is general agreement between our results and those of others. Whilst the values presented in Table 3.1 cannot be presented as firm evidence that the lambs were at basal metabolism, a comparison of these values with those observed during summit metabolism (Tables 3.4, 3.5, 3.6 and 3.7) suggests that the lambs were in a quiescent state when basal metabolism was measured. The respiratory quotient values observed during basal metabolism suggest that approximately equal amounts of carbohydrate and fat were being utilised as energy substrates assuming that protein catabolism was negligible. This finding is similar to that of Alexander (1961) who observed a mean respiratory quotient of 0.80 in lambs maintained at environmental temperatures of $31 - 36^{\circ}\text{C}$.

The behaviour of the lambs during basal metabolism and the stability of blood and plasma composition during prolonged thermoneutral immersion strongly suggests that the metabolic rate recorded was a reliable

estimate of basal metabolic rate and that any increase in metabolic rate which might be observed during cold stress would be a result primarily of that stimulus.

3.2 SUMMIT METABOLISM AT TWO AND FIVE HOURS OF AGE

3.2.1 Introduction

Summit metabolism is defined as 'the highest metabolic rate that can be induced in a resting animal by any cold environment' (Bligh and Johnson, 1973). This definition does not indicate over what time period summit metabolic rate should be assessed. The only proof that summit metabolism has been achieved is the observation of a sustained fall in rectal temperature and since metabolic rate is body temperature dependent the period of measurement of summit metabolic rate is limited in practice to one hour or less (Alexander, 1962b). This raises the question of the value of summit metabolic rate as an index of a lamb's ability to withstand cold stress in the field. Lambs can lose heat rapidly in the first hour or so of life when their coats are soaked with fetal fluids (Sykes, Griffiths and Slee, 1976) but this period of risk is normally rapidly terminated by the ewe which licks the coat dry (Bareham, 1976). Summit metabolic rate may thus be a reliable estimate of the lamb's ability to survive this short period of acute cold stress. However, it is unlikely to be such a reliable index of a lamb's ability to withstand a prolonged period of cold stress such as might occur in bad weather.

In the present study summit metabolic rate was elicited by immersion in progressively cooled water and was measured by open circuit indirect calorimetry. Water was used as a cooling medium in preference to cold air as used by other workers (Alexander, 1961). Water has a very high

specific heat when compared with air and thus it was not necessary to clip the lambs' coats - a procedure which would have created considerable management problems after the experimental period. Cold water is readily available even in warm weather and control of water temperature is technically not difficult. The special apparatus needed was simple and inexpensive to build. The use of water also reduced the need for restraint since even though a support harness was used most of the lamb's weight was supported by the water. Open circuit indirect calorimetry was used in preference to the closed circuit technique for a number of reasons. The apparatus was simple to build and relatively inexpensive. Easy access to the lamb facilitated close observation and blood sampling. The use of high gas flow rates rendered insignificant the existence of small leaks which may not have been detected.

3.2.2 Methods

Summit metabolic rate was estimated at either two or five hours after birth in lambs in which basal metabolic rate had been estimated at either $1\frac{1}{2}$ or $4\frac{1}{2}$ hours (see 3.1). Fasting summit metabolic rate was estimated in 19 Scottish Blackface lambs aged two hours (mean body weight 3.97 ± 0.194 kg, comprising three male singles, five female singles, three male twins and eight female twins) and in 25 Scottish Blackface lambs aged five hours (mean bodyweight 3.97 ± 0.156 kg, comprising four male singles, three female singles, nine male twins and nine female twins). Blood samples were taken from 13 of the younger lambs and 18 of the older lambs.

3.2.3 Results

(a) Behaviour. As the water temperature was progressively lowered the lambs generally became more active. Bleating was common and the lambs

paddled their legs in the water and occasionally shook their heads. During summit metabolism most of this activity ceased.

Shivering was monitored by palpation of the skin on the lamb's back. The onset of shivering was related to metabolic rate by calculating the proportion of the increase in metabolic rate from basal to summit metabolism that had been achieved when shivering was first observed, i.e.:

$$\frac{\text{MR (s)} - \text{BMR}}{\text{SMR} - \text{BMR}} \times 100\%$$

where MR(s) was the metabolic rate observed when shivering commenced, BMR was the basal metabolic rate and SMR was the summit metabolic rate. In the two hour old lambs shivering commenced when $38 \pm 8.0\%$ of the increment in metabolic rate had been achieved ($n = 13$). The corresponding value in the older lambs was $48 \pm 7.9\%$ ($n = 10$). There was no significant difference between these means and the grand mean for all lambs was $42 \pm 5.8\%$ ($n = 23$).

In a few lambs the behaviour described above was less intense or even absent. These lambs generally achieved very low summit metabolic rates.

(b) Metabolic rate. The mean summit metabolic rates in the two groups of lambs were 16.2 ± 0.99 W/kg (231 ± 12.6 W/m²) at two hours ($n = 19$) and 17.0 ± 0.57 W/kg (245 ± 8.0 W/m²) at five hours ($n = 25$). The relationships between summit metabolic rate and body weight were examined by linear and logarithmic regression analysis. The regressions are shown in Table 3.2. The data was then re-analysed but lambs which had shown an uncharacteristic response to cold stress, little or no detectable shivering and little physical activity during cooling, were excluded. The excluded values were 7.2, 7.6 and 14.1 W/kg at two

Table 3.2 The relationship between fasting summit metabolic rate and body weight in 19 Scottish Blackface lambs aged two hours and 25 lambs aged five hours. 'y' represents summit metabolic rate (watts) and 'x' body weight (kg). 'a' and 'b' are constants. The figures in brackets are recalculated values which exclude lambs which showed an atypical response to cold stress (aged two hours n=16 and five hours n=23).

Linear regression: $y = a + bx$

Age (h)	a	b	Variation explained (%)
2	50.34 (26.73)	2.978 (10.07)	3 (36)
5	16.25 (12.11)	12.73 (14.07)	45 (51)

Power regression: $y = ax^b$

Age (h)	a	b	Variation explained (%)
2	52.19 (32.21)	0.1082 (0.5199)	1 (34)
5	25.03 (23.47)	0.7042 (0.7632)	44 (50)

hours of age and 11.8 and 15.0 W/kg at five hours. The recalculated regressions are also shown in Table 3.2. Considering the recalculated logarithmic regressions there was no significant difference between the exponents to which body weight was raised for the two groups of lambs (0.520 and 0.763). The edited results from both groups of lambs were thus combined and a new logarithmic regression calculated:

$$\text{SMR} = 26.76 \text{ BW}^{0.664}$$

where SMR is summit metabolic rate (watts) and BW is lamb body weight (kg). The exponent in this equation of 0.664 to which body weight was raised was significantly different ($P < 0.05$) from 1.00 (summit metabolic rate directly proportional to body weight) but was not significantly different from 0.59 (summit metabolic rate directly proportional to body surface area, Pierce, 1934). The mean rates for summit metabolic rate based on the edited data were 17.5 ± 0.78 W/kg (246 ± 9.4 W/m²) at two hours of age ($n = 16$) and 17.3 ± 0.58 W/kg (248 ± 8.1 W/m²) at five hours ($n = 23$). The mean value for all these lambs ($n = 39$) was 17.4 ± 0.46 W/kg (247 ± 6.1 W/m²).

There were no relationships between summit metabolic rate and litter size, sex or gestational age at birth. There was no relationship between summit metabolic rate and body temperature within the temperature range of 37 to 39°C. Mean summit metabolic rates calculated from both groups of lambs ($n = 44$) at 39.0, 38.5, 38.0, 37.5 and 37.0°C body temperature were 16.1 ± 0.88 , 16.6 ± 1.02 , 15.2 ± 0.95 , 15.7 ± 0.80 and 15.6 ± 0.85 W/kg respectively.

There were no relationships between summit metabolic rate and the duration of the period between the end of basal metabolism and the beginning of summit metabolism, the period of summit metabolism itself or the interval from the end of basal metabolism to the end of summit metabolism.

(c) Respiratory quotient. Three respiratory quotient values were calculated; that during basal metabolism, the value at the metabolic rate calculated for each lamb which was mid-way between basal and summit metabolic rates, and that during summit metabolism. The metabolic rate used in the establishment of the second value was calculated as follows:

$$\frac{\text{SMR} - \text{BMR}}{2} + \text{BMR}$$

where SMR and BMR were the summit and basal metabolic rates respectively. These values for respiratory quotient are shown in Table 3.3. In both groups of lambs a fall in respiratory quotient was recorded between basal metabolism and the mid-way value and this was followed by a rise to the value during summit metabolism, the highest respiratory quotient recorded.

(d) Indices of blood volume. Changes in the mean values of the red blood cell count, the packed cell volume, the blood haemoglobin concentration and the plasma total protein concentration are shown in Table 3.4. In the older lambs there were small but significant increases in the packed cell volume and the blood haemoglobin concentration between basal metabolism and the beginning of summit metabolism. There were similar but not statistically significant changes in the red blood cell count and the plasma total protein concentration. The change in packed cell volume from 39 to 41% could be accounted for by either a 9% increase in total red blood cells, perhaps as a consequence of splenic contraction, an 8% decrease in plasma volume or some combination of these changes. The small increase in plasma total proteins, albeit statistically insignificant, would suggest that some decrease in plasma volume did occur.



Table 3.3 Respiratory quotient (mean \pm SEM) during basal metabolism, at a metabolic rate mid-way between basal and summit metabolisms and during summit metabolism in fasted Scottish Blackface lambs aged either 1½ hours (n=19) or 4½ hours (n=25) at the beginning of basal metabolism (2 and 5 hours respectively at the beginning of summit metabolism). A significant change from one time to another is shown at the latter time.

Age (h)	Respiratory quotient		
	Basal	'Mid-way'	Summit
1½	0.82 \pm 0.015	0.77 \pm 0.017**	0.90 \pm 0.018***
4½	0.86 \pm 0.017	0.81 \pm 0.013**	0.94 \pm 0.013***

(Comparison of basal and summit values 1½h: $P < 0.01$, 4½h: $P < 0.001$)

Table 3.4 Indices of blood and plasma volume (mean \pm SEM) during basal metabolism, and at the beginning and end of summit metabolism in fasted Scottish Blackface lambs aged either $1\frac{1}{2}$ or $4\frac{1}{2}$ hours at the beginning of basal metabolism (2 and 5 hours respectively at the beginning of summit metabolism). A significant change from one time to another is indicated at the latter time.

		<u>$1\frac{1}{2}$ hours</u>		
		Basal Metabolism	Summit Metabolism	
			Beginning	End
Packed cell volume (%)	(n)			
	(13)	40 ± 2.6	40 ± 2.6	40 ± 2.5
Haemoglobin (gl^{-1})	(13)	105 ± 9.9	102 ± 10.1	103 ± 9.9
Plasma total proteins (gl^{-1})	(13)	41.4 ± 2.21	43.1 ± 2.47	42.3 ± 2.30

		<u>$4\frac{1}{2}$ hours</u>		
		Basal Metabolism	Summit Metabolism	
			Beginning	End
Red cell count (10^6 mm^{-3})	(n)			
	(11)	9.04 ± 0.302	9.31 ± 0.367	9.21 ± 0.397
Packed cell volume (%)	(18)	39 ± 1.3	$41 \pm 1.5^{***}$	$40 \pm 1.4^*$
Haemoglobin (gl^{-1})	(18)	132 ± 0.5	$136 \pm 4.7^{***}$	137 ± 1.4
Plasma total proteins (gl^{-1})	(7)	39.1 ± 1.13	40.3 ± 1.13	40.5 ± 0.98

(e) Energy metabolites. Changes in the plasma concentrations of energy metabolites are shown in Table 3.5. Cooling was associated with significant increases in the concentrations of glucose, free fatty acids and glycerol. In both groups of lambs the plasma concentration of glucose increased further during summit metabolism and in the two-hour old lambs only there were significant increases in the concentrations of triglyceride and glycerol. During summit metabolism the plasma glucose concentration in the older lambs was consistently higher than in the younger lambs ($P < 0.05$). The plasma concentrations of β -hydroxybutyrate in the older lambs were higher than those in the younger lambs throughout basal and summit metabolisms ($P < 0.05$). A similar difference, however, was observed in samples taken within five minutes of birth ($39 \pm 7.6 \mu\text{M}$ in the 'younger' lambs, $n = 13$, and $72 \pm 6.1 \mu\text{M}$ in the 'older' lambs, $n = 17$, $P < 0.01$).

(f) Blood gas tensions, acid-base status and indices of tissue hypoxia (Table 3.6). In both groups of lambs there were significant falls in blood pH, base excess and PvO_2 and a significant increase in PvCO_2 in the period between basal metabolism and the beginning of summit metabolism. Further similar changes in blood pH and base excess were observed during summit metabolism. There were significant increases in the concentration of lactate and excess lactate between basal metabolism and the end of summit metabolism in both groups of lambs. Values for excess lactate in the older lambs were higher than those in the younger lambs at both the beginning and the end of summit metabolism ($P < 0.01$).

(g) Plasma hormones (Table 3.7). Cooling was associated with increased plasma concentrations of cortisol and all three catecholamines and a decrease in the plasma concentration of insulin. In the younger lambs a significant reduction in the concentration of tri-iodothyronine was

Table 3.5 Plasma concentrations of energy metabolites (mean \pm SEM) during basal metabolism, and at the beginning and end of summit metabolism in fasted Scottish Blackface lambs aged either 1½ or 4½ hours at the beginning of basal metabolism (2 and 5 hours respectively at the beginning of summit metabolism). A significant change from one time to another is indicated at the later time.

		<u>1½ hours</u>		
		Basal Metabolism	Summit Metabolism	
			Beginning	End
Glucose	(n)			
(mM)	(13)	2.09 \pm 0.395	4.50 \pm 0.395***	5.35 \pm 0.363***
Free fatty acids (mM)	(13)	0.39 \pm 0.040	1.30 \pm 0.134***	1.35 \pm 0.131
Triglycerides (µM)	(13)	130 \pm 21.8	161 \pm 41.2	197 \pm 50.5*
Glycerol (µM)	(13)	148 \pm 16.5	445 \pm 59.0***	570 \pm 80.0**
β-hydroxy-butyrate (µM)	(13)	44 \pm 4.3	40 \pm 6.3	44 \pm 6.6
		<u>4½ hours</u>		
		Basal Metabolism	Summit Metabolism	
			Beginning	End
Glucose	(n)			
(mM)	(17)	3.00 \pm 0.400	5.75 \pm 0.368***	7.17 \pm 0.384***
Free fatty acids (mM)	(17)	0.45 \pm 0.034	1.25 \pm 0.068***	1.23 \pm 0.072
Triglycerides (µM)	(17)	78 \pm 6.7	106 \pm 16.4	144 \pm 21.3
Glycerol (µM)	(17)	104 \pm 37.2	344 \pm 48.6***	423 \pm 49.5
β-hydroxy-butyrate (µM)	(17)	63 \pm 5.7	63 \pm 6.0	64 \pm 6.3
Urea (mM)	(9)	5.36 \pm 0.207	5.14 \pm 0.264	4.75 \pm 0.325

Table 3.6 Blood gas, acid-base status and indices of tissue hypoxia (mean \pm SEM) during basal metabolism and at the beginning and end of summit metabolism in fasted Scottish Blackface lambs aged either 1½ or 4½ hours at the beginning of basal metabolism (2 and 5 hours respectively at the beginning of summit metabolism). A significant change from one time to another is indicated at the latter time.

		<u>1½ hours</u>		
		Basal Metabolism	Summit Metabolism	
	(n)		Beginning	End
Blood pH	(13)	7.33 \pm 0.024	7.24 \pm 0.015***	7.17 \pm 0.018***
PvCO ₂ (kPa)	(13)	7.5 \pm 0.24	8.6 \pm 0.36	9.0 \pm 0.34
Base excess (mEq l ⁻¹)	(13)	3 \pm 2.1	-1 \pm 1.3***	-5 \pm 1.3***
PvO ₂ (kPa)	(13)	5.3 \pm 0.27	3.6 \pm 0.27***	3.8 \pm 0.33
Blood lactate (mM)	(13)	3.85 \pm 0.911	4.57 \pm 0.664***	6.12 \pm 0.713***
Blood pyruvate (μM)	(13)	155 \pm 15.4	159 \pm 14.2	159 \pm 12.6
Blood excess lactate (mM)	(13)	0	0.7 \pm 0.41	2.3 \pm 0.62***
		<u>4½ hours</u>		
		Basal Metabolism	Summit Metabolism	
	(n)		Beginning	End
Blood pH	(18)	7.39 \pm 0.012	7.26 \pm 0.013***	7.20 \pm 0.013***
PvCO ₂ (kPa)	(18)	6.7 \pm 0.19	7.8 \pm 0.34***	8.5 \pm 0.60
Base excess (mEq l ⁻¹)	(18)	5 \pm 1.2	-1 \pm 1.3***	-5 \pm 1.4***
PvO ₂ (kPa)	(18)	5.7 \pm 0.25	4.5 \pm 0.22***	4.7 \pm 0.30
Blood lactate (mM)	(7)	3.50 \pm 0.568	5.10 \pm 0.544**	6.87 \pm 0.554**
Blood pyruvate (μM)	(7)	233 \pm 23.6	220 \pm 25.1	196 \pm 27.7*
Blood excess lactate (mM)	(7)	0	3.1 \pm 0.51	5.0 \pm 0.47**
Plasma lactate (mM)	(11)	3.10 \pm 0.223	7.36 \pm 0.771***	9.39 \pm 0.656***
Plasma pyruvate (μM)	(11)	192 \pm 12.6	211 \pm 14.9	217 \pm 14.1
Plasma excess lactate (mM)	(11)	0	4.1 \pm 0.58	6.0 \pm 0.51**

Table 3.7 Plasma hormone concentrations (mean \pm SEM) during basal metabolism and at the beginning and end of summit metabolism in fasted Scottish Blackface lambs aged either $1\frac{1}{2}$ or $4\frac{1}{2}$ hours at the beginning of basal metabolism (2 and 5 hours respectively at the beginning of summit metabolism). A significant change from one time to another is indicated at the latter time.

		<u>$1\frac{1}{2}$ hours</u>		
		Basal Metabolism	Summit Metabolism	
			Beginning	End
Thyroxine (nM)	(n)			
	(13)	134 ± 13.9	147 ± 11.5	154 ± 15.7
Tri-iodothyronine (nM)	(13)	4.13 ± 0.347	$3.67 \pm 0.281^*$	3.92 ± 0.350
Insulin (pM)	(13)	140 ± 39.1	$60 \pm 10.0^{***}$	50 ± 8.0
Cortisol (nM)	(13)	48 ± 6.1	$126 \pm 11.1^{***}$	$169 \pm 16.2^{**}$
		<u>$4\frac{1}{2}$ hours</u>		
		Basal Metabolism	Summit Metabolism	
			Beginning	End
Adrenaline (nM)	(n)			
	(9)	0.91 ± 0.171	$4.73 \pm 0.928^{***}$	5.44 ± 0.912
Noradrenaline (nM)	(10)	3.82 ± 0.648	$12.56 \pm 1.961^{***}$	12.24 ± 1.367
Dopamine (nM)	(9)	0.87 ± 0.088	$1.50 \pm 0.203^*$	1.72 ± 0.267
Thyroxine (nM)	(7)	145 ± 9.8	164 ± 14.2	167 ± 16.2
Tri-iodothyronine (nM)	(7)	6.31 ± 0.412	5.98 ± 0.555	6.21 ± 0.697
Insulin (pM)	(17)	80 ± 15.1	$30 \pm 6.0^{***}$	30 ± 6.0
Cortisol (nM)	(7)	69 ± 10.8	$127 \pm 18.5^*$	141 ± 23.3

observed between basal metabolism and the start of summit metabolism. The only significant change recorded during summit metabolism was a further increase in the plasma concentration of cortisol in the younger lambs.

The plasma concentration of tri-iodothyronine in the five hour old lambs was consistently higher than that in the younger lambs ($P < 0.01$). Reference to blood samples taken within five minutes of birth showed a similar difference (4.91 ± 0.435 nM, $n = 7$, in the 'older' lambs and 2.80 ± 0.395 nM, $n = 13$, in the 'younger' lambs, $P < 0.01$). During summit metabolism the plasma concentration of insulin was higher in the younger lambs ($P < 0.05$).

(h) Summit metabolic rate and blood composition. Since very similar changes were observed in both groups of lambs and there was no age difference in the rate of summit metabolism the data were pooled for an analysis of the relationship between summit metabolic rate and changes in blood and plasma composition. These relationships were examined by simple linear regression analysis involving the change in metabolite concentration from basal metabolism to the end of summit metabolism and the corresponding change in metabolic rate. The increase in metabolic rate from basal metabolism to summit metabolism was positively correlated with the associated increases in the blood concentration of lactate ($r = 0.742$, $P < 0.001$) and the plasma concentrations of glucose ($r = 0.377$, $P < 0.05$), free fatty acids ($r = 0.454$, $P < 0.05$) and glycerol ($r = 0.617$, $P < 0.001$). The increase in metabolic rate was negatively correlated with the changes in blood pH ($r = -0.482$, $P < 0.001$) and base excess ($r = -0.570$, $P < 0.01$). The change in base excess was negatively correlated with the associated changes in the blood concentration of lactate ($r = -0.645$, $P < 0.01$) and the plasma

concentration of lactate ($r = -0.717$, $P < 0.05$). The only relationship observed between the change in the concentration of a metabolite and the duration of the cold stress was that between the increase in the plasma concentration of glycerol from basal metabolism to the end of summit metabolism and the duration of this period ($r = 0.512$, $P < 0.01$).

(j) Summit metabolic rate and blood composition at birth. Blood and plasma composition at birth, together with the relationships between this and summit metabolic rate, are shown in Table 3.8. Summit metabolic rate was positively correlated with the blood values at birth for pH, base excess and lactate, and was negatively correlated with the blood concentration of pyruvate. Strong correlations were observed between these four values, Table 3.9. Blood pH at birth was positively correlated with that during basal metabolism ($r = 0.843$, $P < 0.001$, $n = 30$), which value was positively correlated with summit metabolic rate ($r = 0.575$, $P < 0.001$, $n = 30$).

3.2.4 Discussion

The major determinant of summit metabolic rate was body weight. Alexander (1962b) found summit metabolic rate to be closely related to body weight raised to the power of 1.0. In the present study summit metabolic rate was best related to body weight raised to the power of 0.7. Heat production capacity in our lambs was thus related more closely to body surface area, to which the rate of heat loss is proportional, than to body weight itself. The significance of this finding is discussed in the General Discussion (3.6) at the end of this chapter.

The supply of oxygen to the heat-producing tissues appeared to be a factor which might limit summit metabolic rate. Heat production

Table 3.8 Blood and Plasma composition in 33 Scottish Blackface lambs within five minutes of birth and the relationships between this and fasting summit metabolic rate (W_m^{-2}) measured at two or five hours of age expressed as coefficients of correlation (r)

	Mean \pm SEM (n)	r
<u>Blood</u>		
Red cell count 10^6 mm^{-3}	9.18 \pm 0.279 (13)	0.223
Packed cell volume %	40 \pm 1.2 (29)	0.079
Haemoglobin gl^{-1}	125 \pm 4.4 (31)	0.278
pH	7.24 \pm 0.019 (32)	0.440*
PvCO ₂ kPa	8.7 \pm 0.27 (30)	0.133
Base excess mEq l^{-1}	-1 \pm 1.1 (32)	0.435*
PvO ₂ kPa	4.4 \pm 0.23 (27)	0.160
Lactate mM	5.67 \pm 0.909 (20)	0.457*
Pyruvate μM	188 \pm 19.6 (19)	-0.480*
<u>Plasma</u>		
Glucose mM	3.02 \pm 0.243 (33)	0.133
Lactate mM	5.10 \pm 0.584 (13)	0.535
Pyruvate μM	126 \pm 12.1 (13)	0.417
Free fatty acids mM	0.49 \pm 0.067 (33)	-0.146
Triglycerides μM	98 \pm 9.2 (33)	-0.223
Glycerol μM	110 \pm 14.9 (33)	-0.080
β -hydroxybutyrate μM	60 \pm 5.6 (31)	-0.058
Total Protein gl^{-1}	4.33 \pm 0.122 (19)	-0.080
Urea mM	5.82 \pm 0.267 (11)	0.015
Adrenaline nM	1.29 \pm 0.547 (9)	0.252
Noradrenaline nM	3.97 \pm 1.072 (10)	0.426
Dopamine nM	1.27 \pm 0.444 (7)	0.689
Thyroxine nM	133 \pm 12.8 (20)	0.128
Tri-iodothyronine nM	3.54 \pm 0.372 (20)	0.227
Insulin pM	91 \pm 9.4	0.260
Cortisol nM	130 \pm 7.5 (20)	0.015

Table 3.9 The relationships between the values in blood for pH, base excess, lactate and pyruvate measured within five minutes of birth in 20 Scottish Blackface lambs expressed as coefficients of correlation.

Base excess	0.845***		
Lactate	-0.896***	0.862***	
Pyruvate	-0.791***	-0.801***	0.858***
	pH	Base Excess	Lactate

was associated with the development of a metabolic acidosis associated with hyperlactaemia. This is indicative of a state of oxygen lack (Huckabee, 1958; Cain, 1977) and the positive correlations between the development of both the acidosis and the hyperlactaemia, and the increase in metabolic rate from basal to summit metabolism indicate that whereas oxygen supply did not restrict heat production in lambs showing low rates of summit metabolism it may have done so in lambs with higher rates. This suggestion is consistent with the findings of Alexander and Williams (1970) who found that summit metabolic rate was depressed by hypoxia (inspired oxygen level of 12 - 13%) but was elevated by up to 12% by hyperoxia (inspired oxygen concentration more than 33%).

Supply of substrate to the heat producing tissues may also have limited summit metabolic rate. The increase in metabolic rate from basal to summit metabolism was positively correlated with the increases in the plasma concentrations of glucose, free fatty acids and glycerol. Whilst substrate supply may not have limited summit metabolic rate in lambs with high rates, it may well have done so in lambs showing low rates. Starvation leads to a depressed capacity for heat production which is associated with hypoglycaemia (Alexander, 1962c).

Similar rates of summit metabolism were observed in the two hour and five hour old lambs. Thermogenic capacity has not been found to increase after five hours of age (Alexander, 1962b) and it appears that this capacity is fully developed within two hours of birth.

A low rate of summit metabolism was associated with a metabolic acidosis and hyperlactaemia at birth. This metabolic state most likely resulted from severe hypoxia encountered during birth (James,

Weisbrot, Prince, Holaday and Apgar, 1958; Comline and Silver, 1972). It thus appears that severe hypoxia during birth is associated with a depressed capacity for heat production for at least the first five hours of life. This depressed summit metabolic rate may have been directly attributable to the acidaemia which was still present when summit metabolic rate was estimated. It is equally plausible that some other lesion caused by severe hypoxia apart from acidaemia was responsible for this. One such factor might be a depression of sympathetic nervous activity. The infusion of catecholamines into newborn lambs under thermoneutral conditions results in rises in the plasma concentrations of glucose, free fatty acids and lactate similar to those seen when a lamb is exposed to cold stress (Alexander, Mills and Scott, 1968). The increase in metabolic rate in our lambs from basal to summit metabolism was positively correlated with the associated increases in the concentrations of these three metabolites. In other words, lambs showing low summit metabolic rates following severe birth hypoxia also showed comparatively small increases in the level of these metabolites. Both these small increases and the low rate of summit metabolism may have been related to depressed sympathetic nervous activity.

Whilst a severe acidaemia might depress summit metabolic rate, the moderate acidaemia which developed during summit metabolism did not (Table 3.6). The progressive development of this acidaemia does, however, suggest that the time for which summit metabolism could be maintained is limited.

The independence of summit metabolic rate from body temperature in the range of 37 - 39°C is in complete agreement with the findings of Alexander in Merino lambs (1962b).

The increases in the plasma levels of glucose, free fatty acids and glycerol associated with increased heat production suggest that both carbohydrate and lipid were utilised as substrates. Values for respiratory quotient during summit metabolism have been previously reported and mean values ranged from 0.81 to 0.85 suggesting that the catabolism of both lipid and carbohydrate was proceeding at approximately equal rates (Alexander, 1962b). Our work has revealed a different picture. In the initial stages of cooling respiratory quotient declined, suggesting that fat catabolism was increasing at a faster rate than was carbohydrate catabolism (Table 3.3). However, respiratory quotients in excess of 0.90 were recorded during summit metabolism suggesting that at this time carbohydrate was the major energy source. The value of 0.94 in the five-hour old lambs indicates that carbohydrate catabolism proceeded at a rate approximately four times higher than that of fat, assuming protein catabolism to be insignificant.

Higher plasma levels of glucose were recorded in the older lambs during summit metabolism. This observation might suggest a greater ability to mobilise carbohydrate reserves in these lambs.

The induction of summit metabolism was associated with changes in plasma hormone levels. The levels of all three catecholamines increased in the period between basal metabolism and the beginning of summit metabolism but there were no significant increases after this time (Table 3.7). It is unlikely that sympathetic nervous activity declined during summit metabolism and it would appear that at the peak levels recorded, the rate of catecholamine release was equalled by the rate of catabolism. The involvement of increased sympathetic nervous activity in the thermogenic response to cold stress in the newborn lamb has previously only been demonstrated

indirectly (Alexander and Williams, 1968; Alexander, 1969; Alexander and Bell, 1982). The present results show directly for the first time that the increase in metabolic rate from basal metabolism to summit metabolism is associated with substantial increases in the plasma concentrations of catecholamines.

The plasma levels of adrenaline and noradrenaline in the lamb would appear to be higher than those in adult ewes (Thompson, Christopherson, Hammond and Hills, 1978). These workers reported mean values for adrenaline and noradrenaline in adult ewes in a warm environment (25°C) of 0.38 nM and 1.42 nM respectively (equivalent values in our lambs were 0.91 nM and 3.82 nM). The mean levels for adrenaline and noradrenaline in adult sheep in a cold environment (-16°C) were 1.37 nM and 6.57 nM respectively (equivalent values in our lambs were 4.73 nM and 12.56 nM).

Plasma levels of adrenaline and noradrenaline have been estimated in a number of species and in common with these species, values for noradrenaline in the lamb were higher than those for adrenaline (Bühler, Da Prada, Haefely and Picotti, 1978; Hansen, Schielke, Jen, Wolfe, Movahed and Pek, 1982).

The plasma levels of corticosteroids in newborn lambs have been previously shown to increase with cold stress (Bassett and Alexander, 1971) and our results support these findings (Table 3.7). The corticosteroids do not seem to be direct determinants of the rate of summit metabolism, the infusion of hydrocortisone into normal lambs during summit metabolism causing no change in metabolic rate (Alexander, 1970). These hormones do, however, play a major role in the heat production process. Adrenalectomy resulted in a depression of summit metabolism to approximately half of control values but

prior treatment with corticosteroids reduced this depression to only 85%. The remaining deficit was restored by the infusion of catecholamines (Alexander and Bell, 1982).

The thermogenic response to cold stress was not associated with any consistent changes in the plasma concentrations of the thyroid hormones, thyroxine and tri-iodothyronine. This finding is in accord with those of other workers who found that the short term infusion of either tri-iodothyronine or thyroid stimulating hormone into lambs exposed to cold stress had no effect on the rate of summit metabolism (Alexander, 1970). The thyroid hormones would appear to exert effects on heat production in an indirect and long term manner. When tri-iodothyronine was administered to lambs over a period of 2-3 days a small but significant increase in the rate of summit metabolism was recorded and when thyroxine was administered to lambs over a 30 day period the normal age-related decline in the rate of summit metabolism was retarded (Alexander, Bell and Williams, 1970). The long term nature of the association between cold stress and changes in the plasma concentration of the thyroid hormones has been demonstrated in the pig (Evans and Ingram, 1977). When young pigs were transferred from a warm environment (32°C) to a cold one (8°C) the maximum increase in the plasma thyroxine concentration was only seen after 24 hours.

In both groups of lambs substantial decreases in the plasma concentration of insulin were observed in the period between basal and summit metabolisms (Table 3.7). At the same time there were substantial increases in the plasma concentration of glucose (Table 3.5). This situation has been previously reported in both newborn and adult sheep (Bassett and Alexander, 1971; Sasaki and Takahashi, 1980) and has been shown to be related to increased circulating catecholamines

which, it is presumed, inhibit insulin secretion. The relationship between glucose and insulin levels in the lamb under cold stress is different from that observed in the lamb at rest where an increase in the plasma concentration of glucose is associated with an increase in the plasma insulin level, presumably due to increased insulin secretion (Alexander, Britton, Cohen and Nixon, 1969; Alexander, Britton, Cohen, Noxon and Parker, 1973). However, further analysis of our results showed that the changes in the plasma concentrations of glucose and insulin were positively correlated ($r = 0.625$, $P < 0.001$) i.e. the lambs showing the highest increases in glucose showed the lowest decreases in insulin, and it would appear that in the lamb under cold stress the rate of insulin secretion is still responsive to some extent to the plasma concentration of glucose.

Overt shivering was observed at a metabolic rate of approximately 2-3 times basal metabolic rate (summit metabolic rate was approximately five times basal metabolic rate). Detection of shivering by palpation is an insensitive technique and it would seem very likely that shivering activity commenced at a metabolic rate of less than this. These observations support the findings of others that shivering thermogenesis is one component of the thermogenic response to cold stress in the newborn lamb (Alexander and Williams, 1968).

The water cooling, indirect calorimetry and recording techniques used in this work proved to be most effective. The use of water permitted a relatively rapid rate of cooling and reduced the duration of exposure of the lamb to cold stress to a minimum. The quiet behaviour of the lambs during summit metabolism and the stable metabolic rate at this time give credence to the rates of summit metabolism recorded. The measurement of gas flow and oxygen levels in the indirect

calorimetry circuit presented few problems but the estimation of carbon dioxide levels was troublesome. Drift in the calibration of the carbon dioxide analyser made frequent calibration checks necessary. The use of a chart recorder as opposed to more sophisticated data-logging devices proved a good choice. Problems such as displacement of a rectal temperature probe were obvious immediately and could easily be remedied.

3.3 SUMMIT METABOLISM AT ONE HOUR OF AGE

3.3.1 Introduction

A low summit metabolic rate was associated with severe birth hypoxia (see 3.2). It seemed reasonable to suggest that this deleterious effect of birth hypoxia would decrease with age and that the best relationship between birth hypoxia and postnatal heat production would be likely to be observed immediately after birth. Thus summit metabolic rate was measured in a group of 21 lambs as soon as possible after birth.

3.3.2 Methods

Fasting summit metabolic rate was estimated in 21 Scottish Blackface lambs (mean weight 3.75 ± 0.107 kg, comprising one male single, one female single, eight male twins and 11 female twins) at an age of 59 ± 2.4 minutes at the beginning of summit metabolism. All procedures were as previously described except that basal metabolism was not measured in order to reduce the period between birth and the estimation of summit metabolic rate to a minimum. Blood samples were taken from all of the lambs.

3.3.3 Results

(a) Metabolic rate. The mean rate of summit metabolism was 18.4 ± 0.48 W/kg (261 ± 7.8 W/m²) ($n = 21$). The relationship between summit metabolic rate and body weight was equally well described by either the linear equation:

$$\text{SMR} = -9.017 + 20.78 \text{ BW}$$

or the logarithmic equation:

$$\text{SMR} = 15.78 \text{ BW}^{1.109}$$

where SMR is summit metabolic rate (watts) and BW body weight (kg). Both equations accounted for 61% of the variation in summit metabolic rate. The exponent in the logarithmic equation of 1.109 was not significantly different from 1.00 but it was significantly different from 0.59 ($P < 0.05$), the exponent relating body weight to body surface area. There were no relationships between summit metabolic rate and sex or gestational age.

(b) Respiratory quotient. Two values for respiratory quotient were calculated, the value during summit metabolism and that mid-way between 'basal metabolism' and summit metabolism. Since basal metabolism had not been measured, the mid-way value was estimated using the mean basal metabolic rate measured previously in the two-hour old lambs. Based on this estimation the respiratory quotient mid-way between 'basal' and summit metabolism was 0.84 ± 0.010 ($n = 21$). The mean value during summit metabolism of 0.94 ± 0.009 ($n = 21$) was again significantly higher ($P < 0.001$).

(c) Blood composition. Values for blood and plasma composition at the beginning and end of summit metabolism are shown in Table 3.10. The changes observed between these two times were very similar both qualitatively and quantitatively to those observed in the lambs aged

Table 3.10 Blood and plasma composition (mean \pm SEM) in 21 fasted Scottish Blackface lambs within five minutes of birth, at the beginning of summit metabolism (mean age 59 ± 2.4 minutes) and at the end of summit metabolism. A significant change during summit metabolism is indicated at the latter time.

	Birth	Summit Metabolism	
		Beginning	End
<u>Blood</u>			
Packed cell volume (%)	40 ± 1.2	39 ± 1.0	37 ± 1.0
pH	7.23 ± 0.012	7.19 ± 0.012	7.14 ± 0.012***
PvCO ₂ (kPa)	9.0 ± 0.27	9.6 ± 0.28	10.1 ± 0.33***
Base excess (m Eq l ⁻¹)	0 ± 0.7	-2 ± 0.8	-4 ± 0.8***
PvO ₂ (kPa)	4.6 ± 0.15	3.8 ± 0.15	3.8 ± 0.16
<u>Plasma</u>			
Glucose (mM)	2.66 ± 0.239	3.83 ± 0.268	5.00 ± 0.311***
Lactate (mM)	6.40 ± 0.580	6.46 ± 0.441	8.02 ± 0.481***
Pyruvate (μM)	155 ± 9.0	121 ± 6.7	136 ± 6.3***
Free fatty acids (mM)	0.44 ± 0.073	1.46 ± 0.057	1.52 ± 0.056*
Glycerol (μM)	97 ± 22.1	597 ± 54.7	755 ± 78.1***
Adrenaline (n=6) (nM)	-	6.7 ± 1.69	9.3 ± 1.55
Noradrenaline (n=6) (nM)	-	20.1 ± 3.82	24.8 ± 3.01
Dopamine (n=6) (nM)	-	2.2 ± 0.21	2.5 ± 0.08
Insulin (pM)	124 ± 23.6	29 ± 2.6	25 ± 2.1**

two and five hours. The plasma levels of catecholamines during summit metabolism appeared to be higher than the levels previously observed in the five-hour old lambs (Table 3.7). The differences were significant for dopamine at the beginning of summit metabolism ($P < 0.01$) and for adrenaline and noradrenaline at the end of summit metabolism ($P < 0.05$ and $P < 0.01$ respectively).

(d) Summit metabolic rate and metabolic state at birth. The relationship between summit metabolic rate and birth hypoxia was examined by a simple regression analysis of summit metabolic rate per unit body weight and the birth values for blood pH and base excess, and plasma lactate. The mean values for these three estimations ($n = 21$) together with the respective coefficients of correlation (r) were:

blood pH:	7.23 ± 0.012	$r = 0.176$
base excess (m equiv/l):	0 ± 0.7	$r = 0.154$
lactate (mM):	6.40 ± 0.580	$r = -0.122$

None of these three regressions was statistically significant.

3.3.4 Discussion

No significant relationships between birth hypoxia and summit metabolic rate were evident in this group of lambs. However, not a single case of severe birth hypoxia was encountered. The most extreme values for pH, base excess and lactate in these lambs were 7.09, -7 m equiv/l and 13.2 mM (plasma) respectively as compared with 6.80, -21 m equiv/l and 19.7 mM (blood) in the previous lambs. They do, however, suggest that if there is an association between birth hypoxia and depressed heat production capacity in early neonatal life that this association is only significant when the hypoxia is severe.

Summit metabolic rate was more closely related to body weight than

to body surface area - apparently the reverse situation of that seen in the older lambs. However, there was no significant difference between the exponent to which body weight was raised in these lambs (1.109) in the logarithmic regression and the corresponding exponent for the older lambs (0.664) and in addition there was no significant difference in body weight.

The relationship between body weight and summit metabolic rate was thus recalculated employing all the data from these lambs and the 'edited' data from the older lambs. The resulting regression was:

$$\text{SMR} = 24.38 \text{ BW}^{0.751} \quad (n = 60)$$

where SMR is summit metabolic rate (watts) and BW body weight (kg). This regression accounted for 43% of the variation in summit metabolic rate. The exponent of 0.751 did not differ significantly from either 1.00 or 0.59. Further discussion of this finding will be found in the General Discussion at the end of this chapter (3.6). The changes in respiratory quotient observed in these young lambs were very similar to those seen earlier in the older lambs.

3.4 SUMMIT METABOLISM AFTER POSTNATAL HYPOXIA

3.4.1 Introduction

The results presented in Section 3.3 made it clear that the further study of the association between hypoxia during birth and summit metabolic rate depended on the artificial exposure of lambs to hypoxia either during or immediately after birth. Although the former alternative was perhaps more likely to recreate the condition observed clinically, the latter was clearly the more feasible and this section describes the effects of an acute postnatal period of hypoxia on the subsequent rate of summit metabolism.

3.4.2 Methods

(a) Animals. The effects of a short period of hypoxia (30 minutes) were investigated in 23 Dorset lambs (mean body weight 4.36 ± 0.127 kg, comprising one male single, three female singles, seven male twins, 10 female twins, one male triplet and one female triplet). The effects of a longer period of hypoxia (three hours) were studied in 17 Scottish Blackface lambs (mean body weight 4.13 ± 0.124 kg, comprising seven male singles, eight female singles and two male twins).

(b) Hypoxia. The lambs were exposed to hypoxia by lowering the inspired concentration of oxygen. In preliminary trials it was found that hypoxia rapidly caused hypothermia, and thus the lambs were placed in warm water at 38.5°C as previously described, before hypoxia was commenced. The face mask was fitted in the normal manner and gas was blown through the mask at a rate of 20 l/min via the original exhaust port. The oxygen tension in the gas mixture was controlled by diluting pumped room air with nitrogen from a cylinder. Both gas flows were monitored using conventional rotameters. The inspired concentration of oxygen was continuously monitored employing the para-magnetic oxygen analyser. The effects of the hypoxia on the lambs were monitored by frequent pH and blood gas analysis of central venous blood, and the oxygen concentration of inspired air was adjusted to cause a fall in the venous base excess value to approximately -20 m equiv/l in a period of either 30 minutes (short period) or three hours (long period). In the 'short period' trials, inspired oxygen concentrations of 2.5 - 3.5% were most commonly found to produce the desired result but in a few lambs it was only necessary to lower the concentration to 5.0 - 7.5%. In the 'long period' trials, oxygen concentrations of 5 - 10% were found to be appropriate. The duration of the hypoxia in the 'long period' trials

varied considerably from lamb to lamb (the hypoxia being terminated when the desired fall in base excess had been observed) with a range of 76 - 265 minutes. The mean duration was 153 ± 16.4 minutes ($n = 12$).

(c) Experimental design. Five treatments were imposed on six groups of lambs (Table 3.11). These treatments were designed to evaluate the effects of two durations of hypoxia, each resulting in the same degree of metabolic acidosis, on the rate of summit metabolism measured either at the end of the period of hypoxia or after a 'recovery' period of 30 minutes. In order to reduce the time lag between the end of hypoxia or 'recovery' period and the elicitation of summit metabolism, the rate of water cooling was increased from the 0.5°C per minute employed previously to 1.0°C per minute. Otherwise the procedures for the estimation of summit metabolic rate were as already described. Blood samples were taken from most of the lambs via indwelling catheters at the end of hypoxia, at the end of the 'recovery' period (if any) and at the beginning of summit metabolism (rectal temperature of 39.0°C). The following estimations were performed: blood values of pH, PvCO_2 , base excess and packed cell volume, and the plasma concentrations of lactate, glucose, potassium and sodium ions, adrenaline, noradrenaline and dopamine.

3.4.3 Results

(a) Behaviour, breathing and heart rate. On immersion in the warm water the lambs behaved as previously described tending to assume a sleep-like state. As the oxygen tension of inspired air was lowered the lambs at first became more active, but then a progressive depression was observed.

In the initial stages of hypoxia the depth of breathing appeared

Table 3.11 Treatments imposed on newborn lambs in the examination of the effects of a period of hypoxia on the subsequently induced summit metabolism.

Breed	n	Age at start of hypoxia (minutes)	Duration of hypoxia (minutes)	Duration of recovery period (minutes)	Age at start of cooling (minutes)
Dorset	7	15	30 (sham) [†]	0	45
	7	15	30	0	45
	9	15	30	30	75
Scottish Blackface	5	165	30	30	225
	7	45	180 (nominal)	0	225 (nominal)
	5	15	180 (nominal)	30	225 (nominal)

[†] Control group. Lambs breathed room air but underwent all experimental procedures.

to increase but as the lambs became depressed the depth and rate of breathing decreased. Towards the end of the period of hypoxia breathing commonly stopped for periods of up to 20 seconds.

The heart rate was monitored manually and after an initial increase in rate a bradycardia developed. In most lambs, towards the end of the period of hypoxia a very slow heart rate of 60 beats per minute or less was recorded. The beat was often irregular. Hypoxia was terminated at this stage when it was considered that the life of the lamb was in danger.

(b) Effects of hypoxia on blood composition (Table 3.12). In the Dorset lambs hypoxia resulted in a profound metabolic acidosis as evidenced by the levels of blood pH and base excess and the plasma lactate concentration. There were dramatic increases in the concentrations of all three catecholamines. There was also a threefold increase in the plasma glucose concentration. There were no significant changes in packed cell volume or in the plasma sodium and potassium ion concentrations.

The blood and plasma picture in the Scottish Blackface lambs at the end of hypoxia was very similar to that seen in the Dorset lambs. The only significant breed difference was a higher plasma adrenaline level in the Scottish Blackface lambs after a short period of hypoxia ($P < 0.05$).

(c) Changes in blood composition during the 30 minute recovery period after hypoxia. In the Dorset lambs (Table 3.13), a partial reversal of the metabolic acidosis was observed at the end of the recovery period. The plasma levels of all three catecholamines fell significantly.

Blood and plasma composition in the Scottish Blackfact lambs at the end of the recovery period was similar to that seen in the Dorset

Table 3.12 Blood and plasma composition (mean \pm SEM) at the end of the period of hypoxia. Significant differences related to length of hypoxia are shown for each breed.

Breed	Dorset		Scottish Blackface	
Duration of Hypoxia (min)	30 (sham)	30	30	180
n	6	16	5	12
<u>Blood</u>				
Packed cell volume (%)	38 \pm 1.0	40 \pm 0.9	42 \pm 2.4	41 \pm 2.3
pH	7.30 \pm 0.040	6.85 \pm 0.028	6.94 \pm 0.049	6.92 \pm 0.033
Base excess m equiv l ⁻¹	3 \pm 0.8	*** -24 \pm 0.7	-21 \pm 1.3	-23 \pm 1.2
<u>Plasma</u>				
Glucose mM	2.24 \pm 0.638	*** 8.11 \pm 0.575	7.72 \pm 0.871	6.83 \pm 1.892
Lactate mM	4.58 \pm 0.660	*** 28.8 \pm 1.22	22.1 \pm 1.41	22.4 \pm 0.95
Adrenaline nM	1.2 \pm 0.39	*** 71.4 \pm 16.03 (n=13)	402 \pm 143	* 216 \pm 121 (n=8)
Noradrenaline nM	3.7 \pm 0.61	*** 105 \pm 21.2 (n=13)	308 \pm 85.4	268 \pm 125 (n=8)
Dopamine nM	1.0 \pm 0.11	*** 44.4 \pm 14.19 (n=13)	82 \pm 27.4	** 18.3 \pm 7.10 (n=8)
Sodium mM	183 \pm 1.0	188 \pm 1.5	185 \pm 2.5	184 \pm 2.5
Potassium mM	5.6 \pm 0.59	6.4 \pm 0.51	6.4 \pm 0.93	6.4 \pm 0.37

Table 3.13 Changes in blood and plasma composition (mean \pm SEM) during the 30 minute recovery period after hypoxia (30 minutes) in nine Dorset lambs. A significant change is indicated at the latter time.

	End of hypoxia	End of recovery
<u>Blood</u>		
Packed cell volume %	42 \pm 1.3	40 \pm 0.8
pH	6.85 \pm 0.037	7.09 \pm 0.019**
Base excess m equiv l ⁻¹	-23 \pm 1.1	-15 \pm 1.6***
<u>Plasma</u>		
Glucose mM	7.79 \pm 0.854	6.36 \pm 0.938
Lactate mM	27.4 \pm 1.76	21.1 \pm 1.16***
Adrenaline nM	101 \pm 28.0	6.3 \pm 1.27**
Noradrenaline nM	132 \pm 37.5	8.6 \pm 1.35**
Dopamine nM	67 \pm 21.5	5.5 \pm 0.71**
Sodium mM	187 \pm 2.4	186 \pm 2.4
Potassium mM	6.9 \pm 0.83	4.9 \pm 0.15

lambs (Table 3.14). The plasma concentrations of adrenaline in the Scottish Blackface lambs at the end of recovery, following a short period of hypoxia, was significantly higher than the corresponding value in the Dorset lambs ($P < 0.05$), a similar situation to that seen at the end of the hypoxic period. The only significant difference in the Scottish Blackface lambs related to the duration of hypoxia was a lower plasma level of glucose in the lambs previously exposed to a long period of hypoxia ($P < 0.01$).

(d) The effects of treatment on summit metabolic rate (Table 3.15). When summit metabolic rate was estimated immediately after a short period of hypoxia in the Dorset lambs, a depression to 66% of the 'sham' value was observed ($P < 0.01$). This depression was not observed after a 30 minute recovery period in either the Dorset lambs or the Scottish Blackface lambs given the same treatment.

When summit metabolic rate was estimated immediately after a long period of hypoxia in Scottish Blackface lambs, a depression to 62% of the value recorded after a short period of hypoxia followed by a recovery was observed ($P < 0.01$). The rate recorded after a long period of hypoxia followed by a recovery period lay mid-way between the other two means for this breed and was significantly different from neither.

(e) Blood composition at the beginning of summit metabolism and relationships with summit metabolic rate. Values for blood and plasma composition at the beginning of summit metabolism are shown in Table 3.16. The most severe metabolic acidosis and the highest levels of catecholamines were found in the lambs not allowed a recovery period after hypoxia. High plasma glucose levels were observed in all groups except the Dorset control lambs ('sham' hypoxia), and the Scottish

Table 3.14 Blood and plasma composition (mean \pm SEM) in Dorset and Scottish Blackface lambs at the end of a 30 minute recovery period after hypoxia.

Breed	Dorset		Scottish Blackface	
Duration of hypoxia (min)	30		30	180 (nominal)
n	5		5	5
<u>Blood</u>				
Packed cell volume %	40 \pm 0.8		42 \pm 2.3	41 \pm 2.1
pH	7.09 \pm 0.019		7.06 \pm 0.027	7.06 \pm 0.023
Base excess m equiv l ⁻¹	-15 \pm 1.6		-18 \pm 1.3	-19 \pm 0.8
<u>Plasma</u>				
Glucose mM	6.36 \pm 0.938		9.41 \pm 1.293	2.90 \pm 0.887
Lactate mM	21.1 \pm 1.16		18.2 \pm 1.11	17.8 \pm 1.01
Adrenaline nM	6.3 \pm 1.27		22.4 \pm 10.33	1.1 [†]
Noradrenaline nM	8.6 \pm 1.35		20.4 \pm 6.81	5.8 [†]
Dopamine nM	5.5 \pm 0.71		9.1 \pm 4.02	2.0 [†]
Sodium mM	186 \pm 2.4		183 \pm 3.7	184 \pm 2.2
Potassium mM	4.9 \pm 0.15		4.9 \pm 0.71	5.3 \pm 0.31

[†] n=1

Table 3.15 The effects of treatment (hypoxia with or without a 30 minute recovery period) on summit metabolic rate.

Breed	n	Duration of hypoxia (minutes)	30 minute recovery period	Summit metabolic rate (Wm^{-2})
Dorset	7	30 (sham)	No	285 ± 13.1
	7	30	No	188 ± 26.0
	9	30	Yes	282 ± 10.9
Scottish Blackface	5	30	Yes	271 ± 5.7
	7	180 (nominal)	No	168 ± 25.4
	5	180 (nominal)	Yes	217 ± 25.3
Scottish Blackface	39	Data from previous studies (see 3.2.3)		247 ± 6.1

Table 3.16 Blood and plasma composition (mean \pm SEM) at the beginning of summit metabolism in six groups of lambs together with the mean rates of summit metabolism. For each breed significant group differences from a reference group are shown. The reference groups (R) are: Dorset, 30 minutes sham hypoxia; Scottish Blackface, 30 minutes hypoxia followed by recovery.

Breed		R		Dorset		R		Scottish Blackface	
Prior Treat- ment	Hypoxia (minutes)	30 (Sham)	30	30	30	30	180 (nominal)	180 (nominal)	
	Recovery period	No	No	Yes	Yes	Yes	Yes	No	No
n		7	7	9	9	5	5	7	7
<u>Blood</u>									
Packed cell volume %		40 ± 1.6	37 ± 0.7	39 ± 1.4		41 ± 2.4	41 ± 2.3	43 ± 3.6	
pH		7.22 ± 0.034	6.92 ± 0.026***	7.11 ± 0.027*		7.12 ± 0.025	7.06 ± 0.027	6.91 ± 0.063*	
Base excess m equiv l ⁻¹		-2 ± 2.2	-23 ± 1.3***	-8 ± 2.2*		-11 ± 1.6	-14 ± 1.6	-22 ± 2.7*	
<u>Plasma</u>									
Glucose mM		3.28 ± 0.269	9.83 ± 1.21***	9.84 ± 0.999***		9.43 ± 1.483	2.83 ± 0.853**	6.22 ± 2.623	
Lactate mM		8.36 ± 0.403	27.4 ± 1.96***	15.3 ± 1.81**		13.4 ± 1.51	13.4 ± 1.98	18.7 ± 2.26	
Adrenaline nM		4.8 ± 0.92	9.4 ± 0.99**	2.4 ± 0.75		7.8 ± 1.68	13.7†	76.9 ± 58.4	
Noradrenaline nM		10.4 ± 0.78	13.2 ± 1.05	5.0 ± 1.78		16.8 ± 3.05	38.9†	69.4 ± 36.7	
Dopamine nM		1.4 ± 0.17	4.0 ± 1.52	2.1 ± 0.67***		4.3 ± 1.02	4.7†	7.0 ± 3.36	
Sodium mM		185 ± 2.1	188 ± 2.2	179 ± 2.7		185 ± 3.1	188 ± 4.9	190 ± 3.2	
Potassium mM		5.9 ± 0.37	5.3 ± 0.24	6.0 ± 0.24		5.5 ± 0.11	5.9 ± 0.14	6.0 ± 0.26	
Summit metabolic rate Wm ⁻²		285 ± 13.1	188 ± 26.0**	282 ± 10.9		271 ± 5.7	217 ± 25.3	168 ± 25.4**	
Cooling time (start of cooling - start of summit metabolism) minutes		26 ± 2.2	14 ± 1.8**	25 ± 1.6		32 ± 1.2	23 ± 1.3***	24 ± 3.9	

† n=1

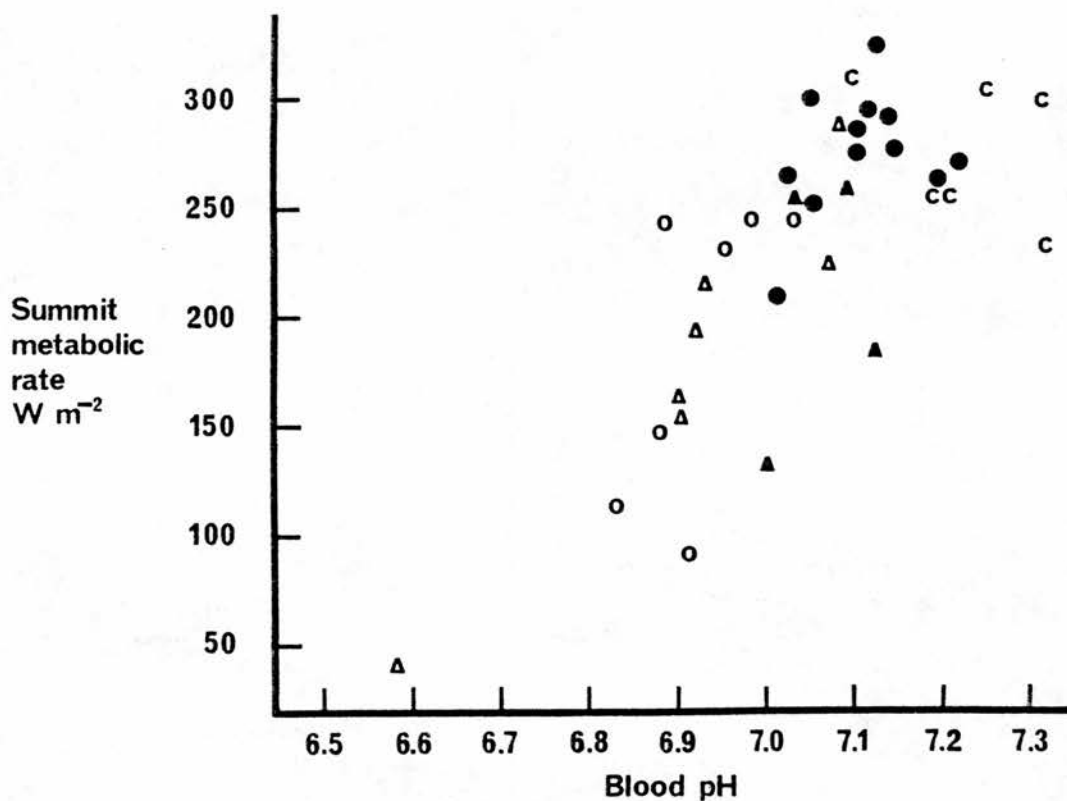
Blackface lambs exposed to a long period of hypoxia followed by a recovery period. There were no significant group differences for packed cell volume or the plasma sodium and potassium ion concentrations. There were no significant differences between the Dorset and Scottish Blackface lambs exposed to a short period of hypoxia followed by a recovery period.

The relationship between blood and plasma composition at the beginning of summit metabolism and summit metabolic rate were investigated by regression analysis (Table 3.17). This analysis was initially conducted on a breed basis. Significant breed differences were identified for the relationships between summit metabolic rate and the blood base excess value and the plasma concentrations of lactate and dopamine ($P < 0.05$) in each case). However, these differences were solely attributable to metabolite concentrations originating from the Dorset lambs not exposed to hypoxia ('sham' hypoxia). When only lambs which had been exposed to hypoxia were considered, no breed differences were identified. There was no evidence for a true breed difference and the analyses were repeated ignoring breed as a variable. The results of this analysis are shown in Table 3.17. Summit metabolic rate was positively correlated with the blood pH and base excess values and was negatively correlated with the plasma lactate concentration, i.e. a severe metabolic acidosis was associated with a low summit metabolic rate. Additionally summit metabolic rate was negatively correlated with the plasma levels of adrenaline and noradrenaline. No other significant correlations were found. The relationship between blood pH and summit metabolic rate is further demonstrated in Figure 3.1. This graphic presentation of the data suggested that the relationship between blood pH and summit metabolic rate was strongest at low pH values and weak or even absent at higher values. This suggestion was investigated by calculating the regressions between blood pH and summit metabolic rate for lambs with

Table 3.17 The relationships between summit metabolic rate and blood and plasma composition at the beginning of summit metabolism in 36 lambs expressed as coefficients of correlation (r).

	n	r	P
<u>Blood</u>			
Packed cell volume	36	0.193	NS
pH	36	0.751	<0.001
Base excess	36	0.698	<0.001
<u>Plasma</u>			
Glucose	36	-0.293	NS
Lactate	36	-0.549	<0.001
Adrenaline	33	-0.671	<0.001
Noradrenaline	33	-0.523	<0.01
Dopamine	33	-0.217	NS
Sodium	36	-0.083	NS
Potassium	36	0.248	NS

NS: not significant



pH values below 7.05 (an estimate of the point of inflection in Figure 3.1) and for those lambs with blood pH values of 7.05 or greater. The results of this analysis together with corresponding regressions for adrenaline and noradrenaline are shown in Table 3.18. Summit metabolic rate was only significantly correlated with blood pH and the plasma level of adrenaline in the lower pH range. In the case of noradrenaline no significant regressions were found. For the lambs in the lower pH range the blood pH and plasma adrenaline levels were negatively correlated ($r = -0.839$, $P < 0.001$, $n = 18$).

3.4.4 Discussion

Exposure to hypoxia resulted in considerable depressions of summit metabolic rate when a recovery period was not allowed (Table 3.15). However, even in this situation, there was a time lag between the end of hypoxia and the start of summit metabolism (Table 3.16) and it would seem likely that heat production capacity at the immediate end of hypoxia would have been lower than the values measured. Hypothetical summit metabolic rates for this time can be extrapolated utilising the mean increase in summit metabolic rate that resulted from a 30 minute recovery period and the mean time period from the end of hypoxia to the beginning of summit metabolism. The extrapolated rate for the Dorset lambs after a short period of hypoxia was 144 W/m^2 and for the Scottish Blackface lambs after a long period of hypoxia was 129 W/m^2 . In both cases heat production capacity would have been approximately halved.

Hypoxia resulted in a severe metabolic acidosis and very high plasma levels of catecholamines (Table 3.12). A low summit metabolic rate was associated with a low blood pH and a high plasma concentration of adrenaline (Table 3.18). Since both the low blood pH and the high plasma adrenaline level resulted from the same insult - hypoxia, it

Table 3.18 The relationship between summit metabolic rate and blood pH, and the plasma concentrations of adrenaline and noradrenaline at the beginning of summit metabolism for two groups of lambs; those with blood pH values less than 7.05 and those with blood pH values of 7.05 or more.

Blood pH at beginning of summit metabolism	n	Summit metabolic rate Mean \pm SEM Range (Wm ⁻²)	Coefficient of correlation (r) between Summit metabolic rate and blood and plasma composition		
			pH	Adrenaline	Noradrenaline
Less than 7.05	18	194 \pm 16.5 41 - 300	0.779***	-0.652**	-0.458
7.05 or more	18	271 \pm 8.0 185 - 325	-0.023	-0.103	-0.260

was not surprising that they were strongly correlated. Either the low pH, the high adrenaline level, both or neither of these factors could have contributed to low summit metabolic rates. A high plasma level of adrenaline does seem a possibility since the infusion of adrenaline at high rates into newborn lambs at summit metabolism caused a decrease in metabolic rate, cardiac output and the blood flows to skeletal muscle, the kidney, the spleen and peri-renal adipose tissue (Alexander, Bell and Setchell, 1972). There is no information on the effects of a low blood pH in lambs but in other species it is associated with central nervous lesions and presumably dysfunction (Dawes, Hibbard and Windle, 1964; Myers, Beard and Adamsons, 1969). A probable role for a low pH is suggested by the finding made earlier, that a low blood pH was associated with a low summit metabolic rate at two hours of age when any high levels of adrenaline present at birth would likely have declined towards 'normal' levels.

The present data do not enable us to differentiate the precise aetiology of the depressed rates of summit metabolism after hypoxia and further work is needed to investigate this problem. Lambs could be exposed to hypoxia as already described but the development of the acidosis prevented by the infusion of either alkali or buffer solution. These techniques would eliminate a low blood pH as a cause of any depressed summit metabolic rate which might be observed.

The lack of any significant change in packed cell volume in these experiments suggests that the effects of hypoxia on summit metabolic rate were not related to any major changes in circulating blood volume. The lack of significant change in the plasma potassium ion level provides no evidence for a significant exchange of extra-cellular hydrogen ions and intra-cellular potassium ions which might have interfered with nervous conduction.

A long period of hypoxia appeared to have a more severe effect on heat production capacity than did a short period (Table 3.15). Even after a 30 minute recovery period full thermogenic capacity had not been regained. This may have been associated with the relatively poor recovery from acidosis in this group compared with those only exposed to a short period of hypoxia (Tables 3.12 and 3.14). It is of interest that handicap in children has been reported as being more likely to follow prolonged partial intrapartum asphyxia than acute periods of complete asphyxia (Scott, 1976).

Care should be taken in the direct extrapolation of these experimental results to the clinical condition of severe hypoxia during birth. Postnatal hypoxia may have different effects from that of birth hypoxia, and whereas the lambs in this study were provided with a warm environment in which to recover, lambs suffering severe hypoxia during birth are faced with an environment which demands an immediate increase in heat production for the maintenance of normothermy. The results presented do, however, support the suggestion made earlier that severe hypoxia during birth is a cause of depressed postnatal heat production capacity and also indicate that the only treatment required in such cases is the maintenance of normothermy until thermogenic capacity is regained.

Severe hypoxia during birth in man is sometimes followed in later life by mental handicap such as cerebral palsy (Scott, 1976). No obvious clinical evidence of 'brain damage' was recorded in these lambs. All of them were returned to their ewes and apparently developed normally. Either no central nervous damage was caused or it was of such limited extent to have been clinically undetectable.

3.5 SUMMIT METABOLISM AFTER FEEDING

3.5.1 Introduction

Alexander (1962b) found that summit metabolic rate in suckled lambs aged 18-28 hours was no higher than the rate measured in the same lambs before sucking at less than six hours of age. There have been no investigations of the effects of feeding in younger lambs. This section describes an investigation into the effects of feeding in the first few hours of life on summit metabolic rate at five hours of age.

3.5.2 Methods

The effects of two types of feeding were investigated; natural sucking from the ewe and bottle feeding.

(a) Natural sucking. 22 Scottish Blackface lambs were used. 11 lambs (mean body weight 4.25 ± 0.239 kg) were allowed to suckle their ewes until four hours of age and a second group of 11 lambs (mean body weight 4.08 ± 0.253 kg) were prevented from sucking as previously described. Basal metabolic rate at $4\frac{1}{2}$ hours of age and summit metabolic rate at 5 hours of age were estimated as before. No blood samples were taken.

(b) Bottle feeding. 31 Scottish Blackface lambs were used. All lambs were removed from their ewes at 30 minutes of age before sucking had commenced. 16 lambs (mean body weight 4.26 ± 0.205 kg) were fed ewe colostrum by bottle at a rate of 830 ml/m² body surface area (approximately 55 ml/kg). The colostrum was obtained in the previous season from nursing ewes one to 28 hours after parturition (mean 20 hours) and was stored in plastic bottles at -20°C. One half of the feed was given at 30 minutes of age and the other half at three hours. The remaining 15 lambs (mean body weight 3.85 ± 0.190 kg) were not fed. Metabolic rates were estimated as previously described. Duplicate 4 ml

blood samples were taken via umbilical vein catheters five minutes after birth, at the end of basal metabolism and at the end of summit metabolism. The following analyses were later performed: packed cell volume, red blood cell count and the plasma concentrations of glucose, free fatty acids, urea, β -hydroxybutyrate and insulin. The colostrum triglyceride, lactose and protein concentrations were estimated.

3.5.3 Results

- (a) Metabolic rate. Feeding resulted in higher basal and summit metabolic rates in both the fed groups (Table 3.19). In the natural sucking experiment basal metabolic rate was elevated by 46% ($P < 0.001$) and summit metabolic rate by 20% ($P < 0.05$); the corresponding increases in the bottle feeding experiment were 34% ($P < 0.01$) and 17% ($P < 0.01$) respectively.
- (b) Respiratory quotient. The respiratory quotient was higher during summit metabolism than during basal metabolism in all the lambs ($P < 0.001$ for each of the four groups) but there were no differences between the fed and unfed groups at any time.
- (c) Blood composition. The plasma levels of glucose and free fatty acids increased two-fold between birth and 4½ hours of age in the fed group ($P < 0.001$ and $P < 0.01$ respectively) but did not change in the unfed group (Table 3.20). Induction of summit metabolism was associated with increases in the concentrations of both metabolites in the fed and unfed lambs, but whereas the plasma glucose level in the fed group was significantly higher than the level in the unfed group during summit metabolism ($P < 0.001$) the plasma free fatty acid level was not ($P < 0.1$). The plasma concentration of insulin was significantly higher in the fed lambs during both basal and summit metabolisms. In both groups of lambs

Table 3.19 Effects of colostrum feeding on metabolic rate and respiratory quotient during basal and summit metabolism in five-hour old lambs: the data is presented as mean \pm SEM.

	Body weight (kg)	Basal metabolism		Summit metabolism	
		Metabolic rate (W/m ²)	Respiratory quotient	Metabolic rate (W/m ²)	Respiratory quotient
<u>Maternal feeding</u>					
Fed lambs (n=11)	4.25 ± 0.239	64 ± 3.2	0.83 ± 0.011	296 ± 11.3	0.90 ± 0.008
Unfed lambs (n=11)	4.08 ± 0.253	44 ± 2.2	0.87 ± 0.022	247 ± 13.5	0.92 ± 0.019
Group difference	NS	P < 0.001	NS	P < 0.05	NS
<u>Bottle feeding</u>					
Fed lambs (n=16)	4.26 ± 0.205	63 ± 2.4	0.88 ± 0.014	283 ± 8.5	0.95 ± 0.009
Unfed lambs (n=16)	3.85 ± 0.190	47 ± 3.9	0.85 ± 0.024	243 ± 9.5	0.96 ± 0.015
Group difference	NS	P < 0.01	NS	P < 0.01	NS

NS: not significant

Table 3.20 Plasma composition (mean \pm SEM) at birth and during basal and summit metabolism in fed (n=13) and unfed (n=11) lambs.

		Birth	Plasma composition	
			Basal metabolism	Summit metabolism
Glucose (mM)	Fed lambs	3.19 \pm 0.364	6.42 \pm 0.490	10.7 \pm 0.55
	Unfed lambs	2.61 \pm 0.317	2.88 \pm 0.577	7.70 \pm 0.549
	Group difference	NS	P < 0.001	P < 0.001
Free fatty acids (mM)	Fed lambs	0.46 \pm 0.091	0.90 \pm 0.126	1.52 \pm 0.117
	Unfed lambs	0.53 \pm 0.133	0.50 \pm 0.048	1.18 \pm 0.116
	Group difference	NS	P < 0.05	NS
Insulin (pM)	Fed lambs	207 \pm 75	840 \pm 189	90 \pm 33.6
	Unfed lambs	81 \pm 15.3	60 \pm 20.9	12 \pm 1.9
	Group difference	NS	P < 0.001	P < 0.001

NS: not significant

a significant fall in the insulin level was observed from basal to summit metabolism ($P < 0.001$ in each case). Neither feeding nor the induction of summit metabolism were associated with any significant changes in the plasma concentrations of β -hydroxybutyrate or urea (mean levels were 0.09 ± 0.01 and 5.6 ± 0.26 mM respectively, $n = 24$).

There were no significant changes in packed cell volume or red blood cell count during the procedures in either group and the group means were not significantly different at any time. The mean values were 41 ± 1.0 per cent and $9.02 \pm 0.151 \times 10^6/\text{mm}^3$ ($n = 24$) respectively.

(d) Colostrum composition. The concentrations of triglyceride, lactose and protein in the colostrum were 118, 42 and 80 g/l respectively.

3.5.4 Discussion

These results clearly demonstrate that feeding would be of immediate survival benefit to the newborn lamb. The increase of nearly 20% in summit metabolic rate (Table 3.19) would result in a reduction of the lower temperature survival limit of the lamb by approximately 10°C (Alexander, 1962a). The depletion of body energy reserves in unfed lambs during the first five hours of life does not lead to a depression of summit metabolic rate (see 3.2.4). Thus the higher summit metabolic rate observed in the fed lambs was related to a specific thermogenic effect of colostrum and not to a repletion of body energy reserves. The absence of a change in packed cell volume or red blood cell count in the fed lambs suggests that the fluid content of the colostrum did not contribute to any expansion of plasma volume and consequently to a possible increase in circulatory efficiency. It seems probable that the energy substrate content of the colostrum was responsible for the increase in summit metabolic rate. When colostrum is fed to the newborn lamb the

plasma concentrations of immunoglobulin and glucose increase within one hour indicating that the ingested colostrum is absorbed quickly from the gut (Comline and Silver, 1972; Mellor and Pearson, 1977). Summit metabolic rate was estimated in the bottle fed lambs 4½ hours after the first feed and two hours after the second and the plasma glucose concentration in the fed lambs during basal metabolism was twice that in the unfed lambs ($P < 0.001$). It is thus reasonable to assume that a significant amount of the ingested energy substrate had been absorbed.

In the bottle feeding experiment, summit metabolic rate in the fed lambs was 40 W/m² higher than in the unfed lambs (Table 3.19). If we assume that this elevation of metabolic rate was present throughout the whole five hour period from birth to the end of summit metabolism, a gross overestimation, we can calculate that the extra energy expended would have been 0.59 MJ/m² body surface area. Analysis of the colostrum showed that the total energy given to the lambs was 5.55 MJ/m² body surface area, 0.60 MJ/m² being derived from carbohydrate, 3.81 MJ/m² from lipid and 1.14 MJ/m² from protein (assuming the calorific values of these substrates to be 17.2, 38.9 and 17.2 kJ/g respectively; White, Handler and Smith, 1964). It is clear that each of the three major substrates in the colostrum was present in an adequate amount to account solely for the increased rate of heat production. In all lambs, during the period from basal to summit metabolism, there was a significant increase in respiratory quotient but there were no significant changes in the plasma levels of urea and β -hydroxybutyrate. These observations and the high respiratory quotient during summit metabolism suggest that carbohydrate catabolism was the major contributor to heat production and that fat and protein catabolism were of limited significance.

The elevation of summit metabolic rate in the fed lambs was associated with plasma levels of both glucose and insulin which were markedly higher than those observed in the unfed lambs. Thus the colostrum-induced elevation of summit metabolic rate could have been a consequence of an increased supply of carbohydrate substrate to the heat producing tissues.

The higher levels of insulin in the fed lambs during summit metabolism confirm the observation made previously, that even when insulin secretion is depressed during cold stress it is still sensitive to the plasma glucose concentration.

The increase in basal metabolic rate observed in the fed lambs is an example of the 'specific dynamic effect' of feed ingestion first identified by Rubner (1902). The exact biochemical mechanism of this effect is uncertain but it would appear that feeding has a similar qualitative effect on heat production in the newborn lamb as it does in the adult sheep (Thompson, Manson, Clarke and Bell, 1978) and in adult and infant humans (Garrow and Hawes, 1972; Alvear and Brooke, 1978).

3.6 GENERAL DISCUSSION

In this section the major findings of this chapter are reviewed. These findings can be summarised as:

1. Body weight accounted for 43% of the variation in summit metabolic rate.
2. Summit metabolic rate was proportional to body weight raised to the power of 0.75.
3. Full thermogenic capacity was achieved within one hour of birth.
4. Dorset Down and Scottish Blackface lambs had similar thermogenic capacities.

5. Exposure to hypoxia, which resulted in a severe metabolic acidosis and very high plasma catecholamine levels, led to a subsequent temporary depression of summit metabolic rate.
6. Carbohydrate appeared to be the major substrate for heat production during summit metabolism.
7. Feeding with colostrum resulted in a marked elevation of summit metabolic rate.

Body weight only accounted for 43% of the variation in summit metabolic rate, even when lambs showing atypical responses to cold stress were excluded from the analysis. This is a marked contrast to observations in 19 Merino lambs aged less than nine hours in which body weight was found to explain 89% of the variation (calculated from Alexander, 1962b). The mean rate of summit metabolism in these Merino lambs of 19.8 ± 0.41 W/kg is significantly greater than the mean value in our Scottish Black-face lambs of 17.8 ± 0.34 W/kg ($n = 60$), $P < 0.01$, and the coefficient of variation is lower, 9% in the Merino lambs and 15% in our lambs. Care must be taken in directly comparing these two sets of data. Apart from differences in breed and age of lamb, different techniques were used for both eliciting and measuring summit metabolic rate. It would appear, however, that we have examined a considerably more variable group of lambs than did Alexander and the origin of this variation is not clear. One possible origin is nutrition during pregnancy. This was controlled in Alexander's work but not in ours, and Alexander has presented some evidence which suggests that poor nutrition during pregnancy may lead to a delayed achievement of full thermogenic capacity in newborn life.

Little confidence can be placed in the precise relationship established between summit metabolic rate and body weight in the Scottish

Blackface lambs. The exponent of 0.75 to which body weight was raised did not differ significantly from 1.00 (summit metabolic rate directly proportional to body weight) or from 0.59 (summit metabolic rate directly proportional to body surface area). This exponent would, however, suggest that the small lamb is not at such a disadvantage in terms of homeothermic capacity when compared with the big lamb, as has been suggested by Alexander (1962b), who found summit metabolic rate to be closely related to body weight raised to the power of one. Heat loss is proportional to body surface area which is itself proportional to body weight raised to the power of 0.59. Thus as the exponent to which body weight is raised in the relationship with summit metabolic rate approaches 0.59 so the homeothermic capacity of the small lamb approaches that of the big lamb. The higher susceptibility of the small lamb to hypothermia (Sykes, Griffiths and Slee, 1976) may be related to a higher rate of heat production per unit body weight and thus a faster exhaustion of body energy reserves which in the small lamb are generally proportionately less than in the big lamb (Alexander, 1974; Mellor, 1983).

The achievement of full thermogenic capacity by one hour of age in the Scottish Blackface lamb is in accord with the finding of Alexander (1962b) who observed no increase in summit metabolic rate from as early as six minutes after birth. This rapid achievement of full thermogenic capacity, which is of obvious benefit to the wet newborn lamb, suggests that circulatory function is not a factor which limits heat production in newborn lambs, since in lambs aged less than six hours both the foramen ovale and ductus arteriosus have not achieved complete functional closure (Alexander and Williams, 1970).

The Scottish Blackface and Dorset Down lambs had similar rates of

summit metabolism. Lambs of the Down breeds and also the Merino are generally found to be more susceptible to hypothermia in the first few hours of life than are lambs of the hill breeds such as the Scottish Blackface (Sykes, Griffiths and Slee, 1976; Slee, Griffiths and Samson, 1980; Samson and Slee, 1981). Since this difference in susceptibility cannot be attributed to differing thermogenic capacities it must be related to a higher rate of heat loss in the Down and Merino lambs, most likely related to the insulation value of the birth coat (Samson and Slee, 1981). The Dorset Down lamb has a 'tight' birth coat, the wool is curled and the skin clearly visible between the 'curls'. In contrast the Scottish Blackface lamb has an even covering of soft straight wool and no skin is visible. It would seem likely that this difference in birth coat, which must be reflected in insulation value, contributes significantly to the differing susceptibilities to hypothermia. An additional 'field' factor might be the activity of the ewe. Whereas the Scottish Blackface ewe generally licks her lamb dry vigorously the Dorset Down ewe is more lethargic in nature and the drying of her lamb's coat may take longer, leading to a greater loss of heat.

Both severe hypoxia during birth, as diagnosed from blood and plasma composition, and induced severe postnatal hypoxia resulted in a depressed rate of summit metabolism. Using the extrapolated minimum rates of summit metabolism for the period immediately after exposure to postnatal hypoxia and the relationships between heat loss and climatic conditions established by Alexander (1962a) it can be calculated that lambs so insulted would become hypothermic in still air at a temperature of approximately 15°C or less. This accords with the clinical observation that affected lambs born inside at an environmental temperature

of 5 - 10°C can become severely hypothermic (<30°C) within 30 minutes of birth. Whilst the precise aetiology of the depression in heat production capacity is not clear it is evident that the depression is transient in nature and that affected lambs recover and develop normally providing the problem is detected and treated appropriately.

During summit metabolism carbohydrate appeared to be the major substrate for heat production, suggested primarily by the high values for respiratory quotient. This observation, which was consistently recorded, is at variance with that of Alexander (1962b) who observed lower values for respiratory quotient, suggesting that fat was catabolised at a faster rate than was carbohydrate. However, the test conditions were totally different and a direct comparison of the results is not valid. The fetal lamb in late pregnancy is predominantly dependent on carbohydrate as its source of energy (Hodgson, Mellor and Field, 1979, 1980 and 1981) whereas respiratory quotient values in the adult ewe under cold stress approach 0.7, indicating that fat is the major substrate for heat production (Thompson, Manson, Clarke and Bell, 1978). The newborn lamb would appear to lie between these two extremes of development. A substantial dependence on carbohydrate for a high rate of heat production would render the lamb highly susceptible to the effects of starvation, for whereas body fat reserves constitute 2-3% of body weight at birth, carbohydrate, stored as glycogen in the liver and in muscle, constitutes only 1% (Shelley, 1960; Alexander, 1962c; Alexander and Bell, 1975). However, at metabolic rates below summit, our observations show that the relative rate of utilisation of fat increases and thus the high susceptibility to starvation related to low body carbohydrate reserves is only likely to be evident when there is a sustained need for a high rate of heat production. Colostrum contains approximately six times more energy in the form of fat

than as carbohydrate and it seems probable that the dependence on carbohydrate as a source of energy is only a temporary feature of newborn life.

Sucking resulted in a 17-20% increase in summit metabolic rate at five hours of age. This finding probably accounts for the comment often made by shepherds that 'a lamb will be fine if it gets a good suck'. The increase in the rate of heat production was associated with higher plasma levels of both glucose and insulin which would increase the availability of glucose to the heat producing tissues. It seems probable that the enhancement of summit metabolic rate was a consequence of a higher rate of carbohydrate catabolism. The change in respiratory quotient which would result from this increase would be practically undetectable.

What then are the implications of the foregoing findings for the aetiology of hypothermia in the field? Before this question is addressed, some assessment must be made of the value of summit metabolic rate as an index of a lamb's ability to resist hypothermia. It is appropriate to state here that summit metabolic rate is an assessment of heat production capacity only and that the techniques used in this work give no information on the determinants of heat loss.

The potential duration of summit metabolism, even assuming no significant fall in body temperature, must be limited. During the ten minute period during which summit metabolism was recorded, significant falls in both blood pH and base excess were observed. A crude estimation of the rates of fall can be made: blood pH, 0.4 units per hour and base excess, 20 m equiv/l per hour. Values for blood pH of below 7.00 were associated with depressed rates of summit metabolism and it seems unlikely that summit metabolism could be maintained for more than one

hour (assuming an initial blood pH value of 7.40). One hour, however, is a period of significant duration for the wet newborn lamb when the rate of heat loss is likely to be greatest. In this first hour the ewe can spend 50% of the time licking the lamb dry (Bareham, 1976) and in most cases there will be a considerable reduction in the rate of heat loss by the end of this period. Summit metabolic rate would thus seem a good estimate of a lamb's ability to withstand hypothermia in the first hour of life, disregarding other factors such as coat insulation value and climate. From this age onwards summit metabolic rate would seem to be a less satisfactory index. The ability to sustain a high but not maximal rate of heat production over a long period would seem to be potentially more important than the ability to maintain a summit rate for a short period. Nutrition would inevitably be one determinant of this ability.

From the present work (Chapter 3) and previous work (Chapter 1) a number of factors which would increase the susceptibility of the newborn lamb to hypothermia can be defined:

1. Low body weight
2. Prematurity
3. Severe hypoxia during birth
4. Poor birth coat with a low insulation value
5. Slowness of the ewe to lick the lamb dry
6. Exposure to wind and rain
7. Slowness of the lamb to suck colostrum
8. Starvation.

The next chapter describes an attempt to estimate the significance of these factors in the aetiology of hypothermia in the field.

CHAPTER IV

THE CAUSES OF HYPOTHERMIA IN NEWBORN LAMBS

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THE CAUSES OF HYPOTHERMIA IN NEWBORN LAMBS

4.1 INTRODUCTION

Despite extensive investigation of the factors which determine the rate of heat loss from, and the rate of heat production by, newborn lambs there is little published information on the causes of hypothermia in the field and of the factors which predispose to it. The prevention and treatment of the clinical condition depend on this information.

This chapter describes two investigations into the causes of hypothermia in newborn lambs. The first is an intensive study of the problem on two farms and the second is a more extensive but less detailed study conducted on 30 farms. This work was conducted in parallel with that described in the next chapter on the detection and treatment of hypothermia.

4.2 INTENSIVE STUDY

4.2.1 Methods

This study was conducted on two farms; 'Cardrona Mains', a lowland farm situated in the Tweed valley near Peebles, and 'Sourhope', a hill farm in the Cheviot Hills. On both farms hypothermic lambs were first identified by the shepherd on the basis of appearance and behaviour and the diagnosis was confirmed by measurement of rectal temperature. A lamb with a temperature of less than 39.0°C was classed as hypothermic. A 2ml blood sample was immediately withdrawn from a jugular vein into a heparinised syringe and the following estimations were performed; packed cell volume and the plasma concentrations of glucose, lactate and immunoglobulin G. The lamb was weighed, examined and a brief

history noted including possible exposure, age, type (single, twin, etc.) and breed. Treatment as outlined in Chapter 5 was then commenced. Postmortem examinations were performed on 15 of the 32 lambs which died during or soon after treatment. Brain, spinal cord and portions of liver and lung were removed from all carcasses and placed in Baker's fixative together with any other tissues showing gross abnormality. Selected blocks of these were processed for light microscopy. Paraffin sections (12 μ m) from brain and spinal cord were stained by the Luxol fast blue method and frozen sections (20 μ m) by the osmium tetroxide-alpha naphthylamine method. Paraffin sections of all tissues (6 μ m) were stained by haematoxylin and eosin and of liver alone by the periodic acid-Schiff method.

4.2.2 Results

(a) History, examination and clinical chemistry. The 89 lambs comprised 36 Suffolk crosses, 33 Cheviots, 14 Scottish Blackface, four Texel crosses, one Border Leicester cross and one Dorset cross. Seven of the lambs were singles, 57 were twins, 23 were triplets and two were quads. The mean size of litter from which the lambs came was 2.2 lambs. The mean litter size for all the lambs born in the two flocks was about 1.4 lambs. The mean body weight of the hypothermic lambs was 3.2 ± 0.09 kg with a range from 1.3 to 5.2 kg.

The distribution of the lambs according to age at presentation is shown in Figure 4.1. 35 lambs (39%) were presented within four hours of birth, 16 lambs (18%) at 4-16 hours, 29 lambs (33%) at 16-48 hours and nine lambs (10%) at more than 48 hours.

The mean rectal temperature on presentation was $29.6 \pm 0.52^{\circ}\text{C}$ ($n = 89$) with a range of $16.0 - 37.5^{\circ}\text{C}$. In 42 cases (47%) rectal

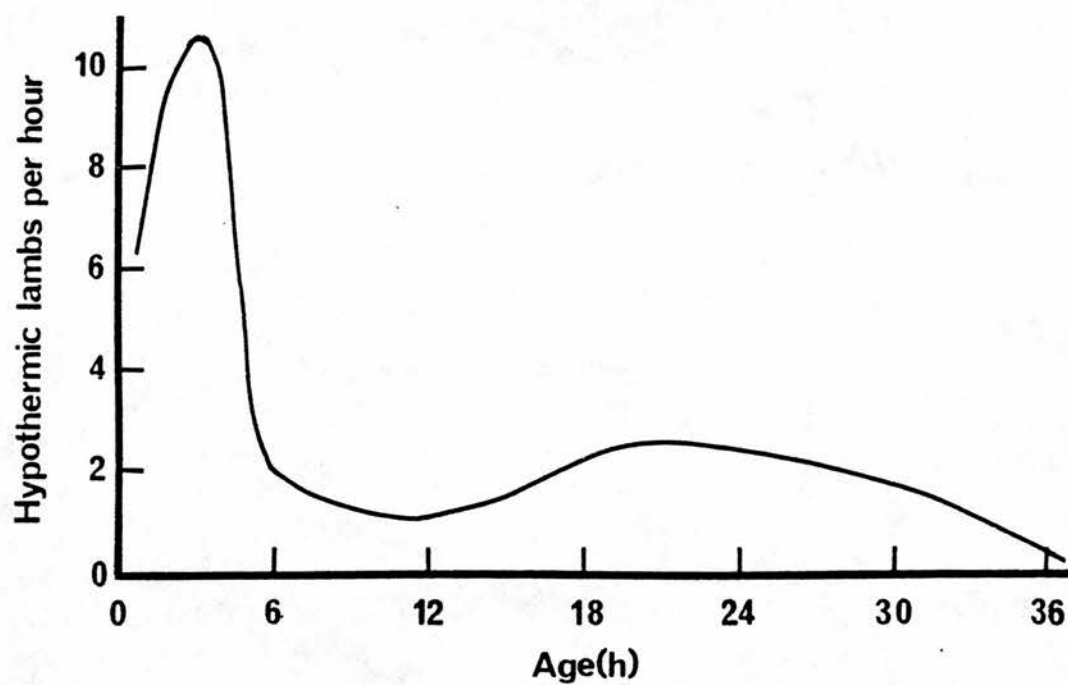


Figure 4.1 The distribution of the hypothermic lambs according to age (intensive study).

temperature was below 30.0°C. Only three lambs were presented with temperatures in the range of 37.0 - 39.0°C.

The appearance and behaviour of the lambs was related to both age and rectal temperature (Table 4.1). In general the lower the temperature and the older the lamb, the worse was its physical condition.

The plasma concentrations of glucose, lactate and immunoglobulin G are shown related to age at presentation in Figure 4.2. High values for the plasma concentrations of glucose and lactate were observed in lambs which became hypothermic in the first few hours of life but as age at presentation increased so these values decreased. Very low plasma immunoglobulin G levels were observed in all the lambs. The mean packed cell volume was $46 \pm 0.4\%$ ($n = 75$).

Two lambs, both aged less than two hours, had multiple rib fractures, one lamb aged 24 hours was affected by acute phenol poisoning (Eales, Small, Oliver and Quigley, 1981) and another aged more than 48 hours was presented with gross abdominal distension which on postmortem after humane destruction was found to be caused by an intestinal obstruction.

(b) Diagnosis of cause of hypothermia. On the basis of history, examination and clinical biochemistry an attempt was made to diagnose the cause of hypothermia in each case. Excessive heat loss was diagnosed as the cause in lambs with a history of exposure, a normal plasma concentration of immunoglobulin G (related to age), but elevated levels of glucose and lactate indicating a thermogenic response to a cold stress. Depressed heat production consequent to severe hypoxia during birth was diagnosed as the cause in a lamb aged less than 12 hours which showed a basal plasma concentration of glucose suggesting a poor thermogenic response to cold stress but a high

Table 4.1 The appearance and behaviour of the lambs according to age and rectal temperature.

Appearance and behaviour					
Age (hours)	37°C	35°C	30°C	25°C	20°C
0 - 5	Able to walk but activity depressed	Weak but could stand	Recumbent	Coma	Deep coma
> 12	Weak but could stand	Recumbent	Coma		

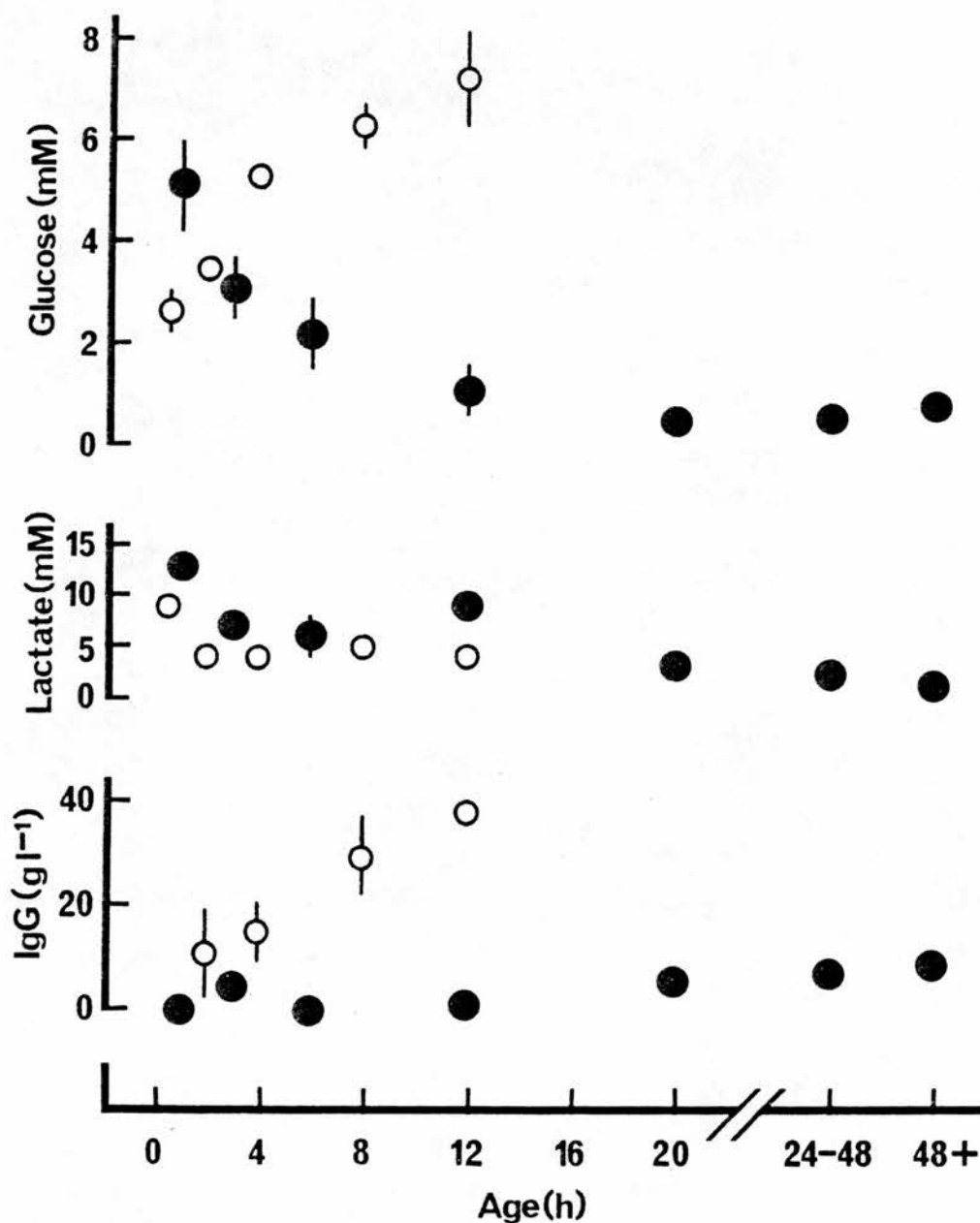


Figure 4.2 The plasma concentrations (mean \pm SEM) of glucose, lactate and immunoglobulin G (IgG) in 79 hypothermic lambs (intensive study) related to the ages at which they became hypothermic. The open circles show mean values from 18 healthy well fed Scottish Blackface lambs (Mellor and Pearson, 1977).

concentration of lactate indicative of a severe hypoxic episode.

Depressed heat production due to starvation was diagnosed as the cause in a lamb aged more than six hours which had a low plasma concentration of immunoglobulin G indicative of a low intake of colostrum (Shubber, Doxey, Black and FitzSimons, 1979), and low plasma levels of both glucose and lactate. A summary of the criteria used is shown in Table 4.2.

Employing these criteria a positive diagnosis was arrived at in 77 cases (Table 4.3). Excessive heat loss accounted for 22 cases (25%) and was mainly a characteristic of the first four hours of life. Severe hypoxia during birth accounted for nine cases (10%), mostly occurring in the first two hours of life. Starvation accounted for 42 cases (47%) and was found mainly in lambs aged 16 hours or more.

No diagnosis was made in 12 cases (Table 4.4). These 12 lambs had three factors in common. They were all aged less than eight hours, they all had plasma glucose concentrations of 2.0 mM or less and none of them showed a high plasma level of lactate. Low plasma levels of both glucose and lactate in a young lamb suggest a poor thermogenic response to cold stress in the first few hours of life. In the absence of evidence of severe hypoxia during birth some form of immaturity would seem a likely explanation for this. Immaturity in terms of heat production capacity is known to be associated with prematurity (Dawes and Parry, 1965; Alexander, Thorburn, Nicol and Bell, 1972). It would also be a likely result of any restriction to fetal development which might be evidenced by low birth weight and/or a high packed cell volume consequent to chronic fetal hypoxia (Mellor and Pearson, 1977; Robinson, Kingston, Jones and Thorburn, 1979). Of these 12 lambs four were of body weight below 2.5 kg, four had packed cell volume values

Table 4.2 Criteria employed in the diagnosis of cause of hypothermia on the basis of history and clinical chemistry.

<u>Diagnosis</u>	<u>History</u>	<u>Clinical Biochemistry</u>
Excessive heat loss	Exposure	Plasma glucose > 3.0 mM Plasma lactate > 6.0 mM Plasma immunoglobulin G appropriate for age.
Depressed heat production caused by:		
Severe hypoxia during birth	Age < 12 h	Plasma glucose < 4.0 mM Plasma lactate > 10.0 mM
Starvation	Age > 6 h	Plasma glucose < 2.0 mM Plasma lactate < 6.0 mM Plasma immunoglobulin G low for age.

Table 4.3 Distribution of hypothermic lambs according to age and cause of hypothermia diagnosed according to the criteria shown in Table 4.2.

<u>Diagnosis</u>	Lamb age (hours)							All ages
	0 - 2	2 - 4	4 - 8	8 - 16	16 - 24	24 - 48	>48	
Excessive heat loss	6	11	3	1	1	0	0	22
Depressed heat production caused by:								
Severe hypoxia during birth	6	2	0	1	0	0	0	9
Starvation	0	0	0	7	17	10	8	42
Other causes	2	0	0	0	1	0	1	4
Unknown	1	7	4	0	0	0	0	12
All causes	15	20	7	9	19	10	9	89

Table 4.4 History and plasma composition in 12 lambs in which the cause of hypothermia had not been diagnosed (Table 4.3).

No	History			Packed cell volume and plasma composition				
	Age hours	Weight kg	Litter size	Known prematurity	Packed cell volume %	Glucose mM	Lactate mM	Immunoglobulin G g l ⁻¹
79/13	4	3.5	2		53	0.9	0.9	<1
79/17	2	2.3	2		50	0.3	0.8	5.7
79/21	2	1.3	2		61	0.5	1.7	<1
79/27	6	1.9	2		56	2.0	4.5	<1
80/1	5	3.4	2		44	0.8	2.5	<1
80/18	3	3.9	2		43	0.3	1.5	4.7
80/25	2	4.4	3		37	0.8	2.2	11.3
80/26	2	3.0	3		40	0.4	2.6	<1
80/27	2	3.0	3		36	0.6	2.5	9.6
80/28	2	3.4	2		31	0.5	4.5	18.4
80/101	1	3.2	2	Yes	35	1.7	3.5	<1
80/107	4	2.0	2		56	1.7	5.7	<1
Mean	3	2.9	2.3		45	0.9	2.7	4.7
SEM	0.5	0.26	-		2.8	0.16	0.44	1.63

in excess of 50% and one at least was premature (gestational age less than 140 days). On the basis of all the evidence presented, a diagnosis of excessive heat loss predisposed by immaturity is suggested for this group of lambs (Table 4.5).

(c) Postmortem findings. A summary of the postmortem findings classified according to the diagnosis made in Table 4.5 is shown in Table 4.6. Three specific neurological abnormalities were found. In five lambs non-myelinated areas were found in the corpus callosum, cerebral gyri and the folian cores of the vermis where myelination should have commenced by 140 days of gestation (Romanes, 1947; Barlow, 1969). These lambs were either premature or they were born after 140 days of gestation, but myelination was delayed. Porencephaly or white matter cavitation which reflects some insult to the fetus during development was found in two lambs and periventricular sinuses, the significance of which is unknown, were found in two lambs. Retention of fetal lung characteristics which is evidence for immaturity (Reynolds and Strang, 1966) was found in five lambs.

The finding of histological evidence of immaturity in two lambs from the 'excessive heat loss predisposed by immaturity' group supports this diagnosis. However, the finding of histological evidence of immaturity in lambs from the 'starvation' group suggested the possibility that immaturity may have been a predisposing factor to hypothermia in some of these lambs and perhaps in some of the lambs in which severe hypoxia during birth had been diagnosed. The data was thus reviewed and immaturity was identified as a factor predisposing to hypothermia in any lamb which was either known to be premature (gestational age less than 140 days), showed histological evidence of immaturity, had a packed cell volume value in excess of 50% or

Table 4.5 Revised distribution of hypothermic lambs according to age and cause of hypothermia including excessive heat loss predisposed by immaturity as a diagnosis.

Diagnosis	Lamb age (hours)							All ages
	0 - 2	2 - 4	4 - 8	8 - 16	16 - 24	24 - 48	>48	
Excessive heat loss	6	11	3	1	1	0	0	22
Excessive + heat loss (predisposed by immaturity)	1	7	4	0	0	0	0	12
Depressed heat production caused by:								
Severe hypoxia during birth	6	2	0	1	0	0	0	9
Starvation	0	0	0	7	17	10	8	42
Other causes	2	0	0	0	1	0	1	4
All causes	15	20	7	9	19	10	9	89

+ This diagnosis includes the 12 lambs designated as 'unknown' in Table 4.3 and listed in detail in Table 4.4.

Table 4.6 Distribution of neuropathological changes and retention of fetal lung characteristics in 15 lambs according to diagnosis of cause of hypothermia shown in Table 4.5.

Diagnosis	n	Non-myelination	Porencephaly	Periventricular sinus dilation	Retention of fetal characteristics in lungs
Excessive heat loss predisposed by immaturity	2	2	1	1	1
Depressed heat production caused by:					
Severe hypoxia during birth	1	0	0	0	0
Starvation	10	3	1	1	4
Other causes	2	0	0	0	0

or weighed less than 2.0 kg. The results of this review are shown in Table 4.7. Immaturity was found to be a factor predisposing to hypothermia in 31 cases (35%).

4.3. EXTENSIVE STUDY

4.3.1 Methods

The major aim of this study was to test the techniques for the detection and treatment of hypothermia described in Chapter 5 which had been developed in parallel with the intensive study described above. However, some data relevant to this chapter was recorded.

Thirty farms situated throughout Scotland were involved. Each farm was visited before lambing and the shepherd requested to complete a questionnaire for each lamb that became hypothermic. The questionnaire was stamped into the pages of a pocket note-book and requested the following information relevant to this chapter.

For the lamb:

Type (single, twin, triplet or quad)

Age (0-5, 5-12, 12-24 or more than 24 hours)

Temperature (37-39°C or less than 37°C)

For the lamb's dam

Age (1, 2, 3-5 or 6 years).

The shepherd was provided with a novel electronic thermometer for recording rectal temperature (Chapter 5). This instrument indicated by means of different coloured flashing lights whether the lamb's temperature was more than 39.0°C, 37.0-39.0°C or below 37.0°C.

4.3.2 Results

493 hypothermic lambs were identified, 2.6% of all the lambs born

Table 4.7 Distribution of hypothermic lambs according to age and cause of hypothermia. The number of lambs in which immaturity was judged to be a predisposing factor is given in brackets.

Diagnosis	Lamb age (hours)							
	0 - 2	2 - 4	4 - 8	8 - 16	16 - 24	24 - 48	>48	All ages
Excessive heat loss	7(2)	18(8)	7(4)	1	1	0	0	34(14)
Depressed heat production caused by:								
Severe hypoxia during birth	6	2	0	1	0	0	0	9(0)
Starvation	0	0	0	7(4)	17(9)	10(3)	8(1)	42(17)
Other causes	2	0	0	0	1	0	1	4(0)
All causes	15(2)	20(8)	7(4)	9(4)	19(9)	10(3)	9(1)	89(31)

on the 30 farms. The incidence of hypothermia varied greatly from farm to farm with a range of 0.5 - 13% of lambs born alive.

The distributions of the lambs according to type, age and rectal temperature are shown in Tables 4.8, 4.9 and 4.10 respectively. Hypothermia was six times more frequent in twins than in single lambs, and in triplets 13 times more frequent than in single lambs. Most cases of hypothermia were recorded either in the first five hours of life (46%) or after 12 hours of age (48%). Only 6% of cases occurred at 5-12 hours. Nearly one third of the cases were detected before rectal temperature had fallen below 37.0°C.

The distribution of the lambs according to age of dam is shown in Table 4.11. The number of ewes of each age group which lambled is unknown as is the average litter size in each age group, and thus these figures are very difficult to interpret. The apparent low incidence rates from ewes aged either one year or six or more years are almost certainly related to lower numbers of ewes of these ages. All that can be said with confidence is that lambs from ewes of all ages are susceptible to hypothermia.

4.4 DISCUSSION

Death due to hypothermia has until now normally been attributed to the 'starvation/exposure syndrome' (Johnston, 1977). The evidence presented in this chapter demonstrates that this diagnosis would be inappropriate in most, if not all, of the cases described. The causes of hypothermia in the first few hours of life were excessive heat loss in the majority of cases and in a minority, depressed heat production consequent to severe hypoxia during birth. In lambs aged more than 12 hours starvation was the major cause. Very few cases occurred in the age period 5-12 hours

Table 4.8 Extensive study - distribution of the hypothermic lambs according to type.

	Hypothermic lambs	Estimate of all live lambs born	Incidence of hypothermia per 1000 lambs born alive
Singles	49 (10%)	7,760 (43%)	6
Twins	306 (65%)	8,843 (49%)	35
Triplets+	115 (25%)	1,444 (8%)	80
Total	470 (100%)	18,047 (100%)	26
Unknown	23		

Table 4.9 Extensive study - distribution of the hypothermic lambs according to age.

Age when hypothermia was detected (hours)	Lambs
0 - 5	200 (46%)
5 - 12	26 (6%)
12 - 24	77 (18%)
More than 24	137 (30%)
<hr/>	
Total	440 (100%)
Unknown	53

Table 4.10 Extensive study - rectal temperature at time when hypothermia was detected.

Temperature °C	Lambs
37 - 39	133 (27%)
Less than 37	360 (73%)
<hr/>	
Total	493 (100%)

Table 4.11 Extensive study - distribution of the hypothermic lambs according to age of dam.

Age of dam (years)	Hypothermic lambs
1	21 (5%)
2	105 (24%)
3 - 5	263 (60%)
6 or more	50 (11%)
<hr/>	
Total	440 (100%)
Unknown	53

when presumably the risk of excessive heat loss associated with a wet birth coat had passed but body energy reserves had not been exhausted. If uncomplicated excessive heat loss had been a significant cause of hypothermia in the older lamb, we would have expected to have identified at least one older lamb which showed no evidence of starvation. No such lamb was found nor has been found in subsequent investigations. It is not insignificant that more than half of the cases of hypothermia in the older lambs recorded in the extensive study occurred in housed situations.

Immaturity was judged to have been a predisposing factor to hypothermia in 35% of the cases recorded in the intensive study. Whilst the nature of this study necessitates that a large error be attached to this figure, perhaps $\pm 10\%$, the figure is sufficiently high to enable us to say with some confidence that immaturity was a significant factor. This is not surprising. Hypothermia is recognised as a common complication of immaturity in the newborn baby (Forfar and Arneil, 1978). The higher incidence of hypothermia in twins and triplets supports this finding since it is these lambs that are most likely to either have been born prematurely or to have experienced some restriction to development in utero related either to small placental size or maternal undernutrition (Alexander, 1974; Mellor and Pearson, 1977; Black, 1983; Mellor, 1983).

There are further factors which would increase the susceptibility of twins and triplets to hypothermia. The newly lambed ewe will take longer to lick twins or triplets dry than she would a single lamb and heat loss will thus be greater. Twins and triplets take longer to stand and suck than single lambs (Bareham, 1976) and thus the increase in heat production capacity that early sucking confers will be delayed.

Twins and triplets have a higher rate of heat production per unit body weight than does the larger single lamb and thus energy reserves will be exhausted faster. The milk requirement of a set of twins or triplets considerably exceeds that of a single lamb and thus starvation is more likely.

The newborn lamb and especially the very young lamb appeared physically remarkably normal in the early stages of hypothermia. This made early detection, which is desirable from the point of view of treatment, a considerable problem. This subject will receive more attention in the next chapter.

In the extensive study, hypothermia was recorded in 2.6% of all lambs born alive. This figure was somewhat below that expected (Eales, Small and Gilmour, 1983). Two factors probably accounted for this. First, exceptionally good weather was experienced in this season (1981) and second, some cases were missed. However, even if this low rate of incidence is taken as representative of the national situation it can be calculated that the number of lambs affected by hypothermia annually in the United Kingdom is in the region of 500,000.

Three findings emerge from our observations which have application to the prevention of hypothermia. First, if most cases occur within a few hours of birth excessive heat loss is likely to be the major cause and more shelter and attention to newborn lambs, especially twins and triplets, is required. Second, if most cases occur in lambs aged 12 hours or more, starvation is likely to be the major cause and attention should be directed to the nutrition of the lambs and the ewes. Finally it is inevitable that some lambs will be born with poor thermoregulatory ability and these lambs must be provided with adequate shelter and nutrition.

Under ideal conditions it is possible to prevent hypothermia in most cases. However, ideal conditions in terms of both facilities and labour are rarely found. Hypothermia is thus inevitable and effective techniques for early detection and treatment are required. The search for these techniques is the subject of the next chapter.

CHAPTER V

THE DETECTION AND TREATMENT OF HYPOTHERMIA IN NEWBORN LAMBS

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THE DETECTION AND TREATMENT OF HYPOTHERMIA IN NEWBORN LAMBS

5.1 INTRODUCTION

The aim of the work described in this chapter was the development of improved on-farm methods for the detection and treatment of hypothermia in newborn lambs based on the understanding of the condition gained from the work described in the preceding chapters and from previously published work. In the past the treatment of hypothermia was a rather haphazard affair and in many cases it was not successful. This lack of success was often unrecognised since, whereas successful resuscitation was credited to the efficacy of the techniques used, lack of success was often ascribed to 'the patient being beyond treatment', 'Acts of God' and the like. This work was undertaken in two phases in parallel with the studies described in Chapter 4. The first phase was the development of the techniques and associated equipment which was conducted on the two farms mentioned in Chapter 4 (Intensive Study) and the second phase was an extensive field trial of these techniques on 30 commercial farms.

5.2 DEVELOPMENT

5.2.1 Methods

(a) Lambs. The lambs treated were the 89 lambs described in the Intensive Study in Chapter 4 (see 4.2).

(b) Detection of hypothermia. No special techniques were employed. The lambs were identified as hypothermic by the shepherd on the basis of behaviour and appearance and the diagnosis was later confirmed by measurement of rectal temperature.

(c) Treatment of hypothermia.

(1) Drying. Initially no attempt was made to dry lambs before warming. However, it was often found that for the first 30 minutes or so of warming, the lamb's temperature fell instead of rising. This fall was attributed to heat loss caused through the evaporation of water from the lamb's coat. Subsequent lambs were thus dried with a towel before warming and this drop in rectal temperature was avoided.

(2) Warming. The lambs were warmed in air at 40-45°C. Warm air was used in preference to infra-red lamps, commonly used to warm hypothermic lambs on farms, since the temperature is easy to monitor and control and there is no risk of skin burns. In the early stages of the trial this environment was obtained using converted cabinet clothes-driers powered by mains electricity (Fig. 5.1). The temperature was controlled by means of a proportional temperature control unit which controlled the energy supply to the heater in proportion to the deviation of the heater temperature from the desired temperature. Though effective, this warmer was not practical for three reasons; it was not readily available, each heater could only hold about four lambs and each time a lamb urinated a short circuit was created which 'tripped' a circuit breaker. Thus the bale warmer was developed (Fig. 5.2). This consisted of a small pen, measuring externally 7 feet (2.1m) square, made of horizontally laid straw bales, two bales high. The pen was divided horizontally into two chambers by a sheet of half inch (1.3cm) weld mesh and a steel tunnel was placed in a gap in the bottom layer of bales. Warm air was blown into the lower chamber from a 3kW domestic fan heater positioned in the steel tunnel. A sheet of polythene was fitted over the top of the whole assembly to retain heat. The air temperature in the upper chamber was controlled either

Figure 5.1 A 'clothes-drier' lamb warmer with temperature control unit.

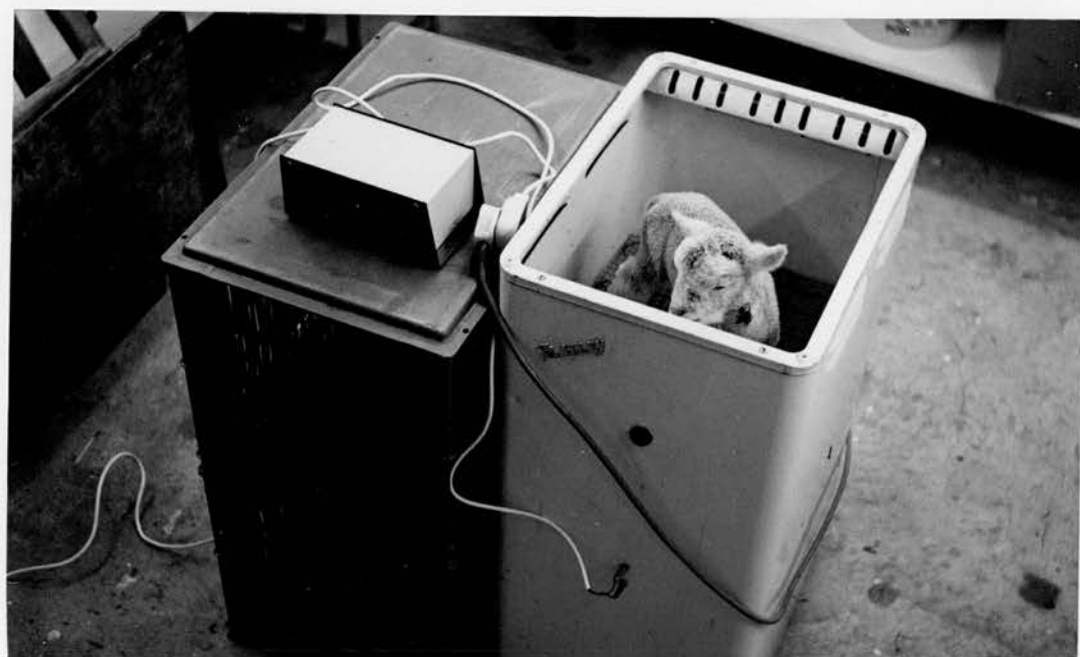
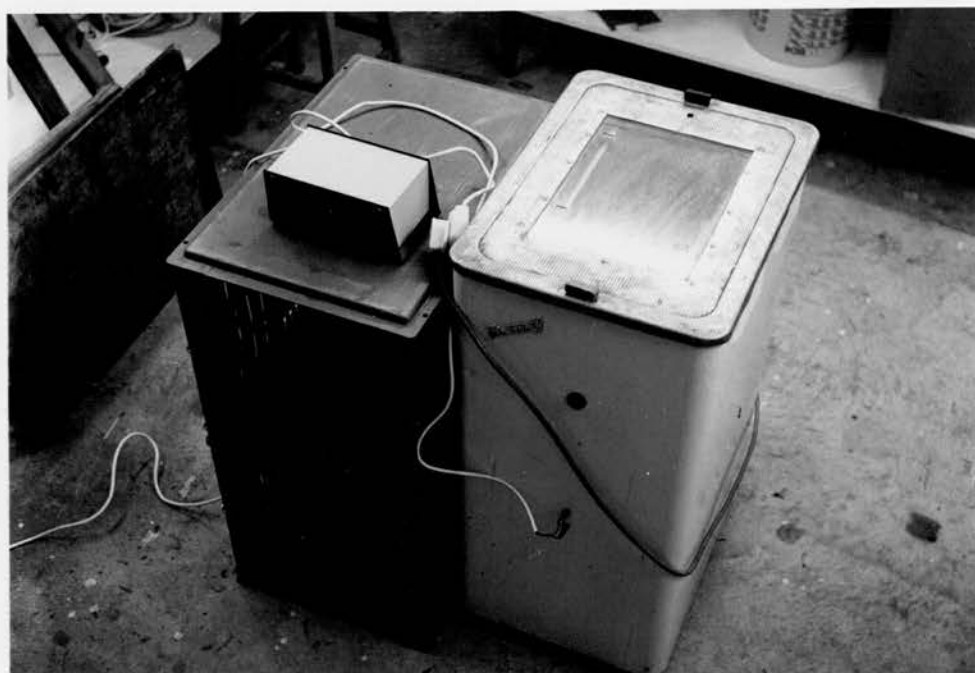


Figure 5.2 A bale lamb warmer in three stages of construction.



by adjusting the kilowatt setting on the heater, by adjusting the position of the heater in the tunnel or by cutting a ventilation hole in the plastic sheet. The lamb was placed on top of the weld mesh under the plastic sheet and was removed when rectal temperature had risen to 38.0°C.

(3) Glucose Injection. A single intraperitoneal injection of glucose solution was given to 64 lambs before rewarming to investigate the efficacy of this for the reversal of hypoglycaemia (Fig. 5.3). A 50 ml syringe fitted with a 19 gauge 1 inch (2.6 cm) needle was used. The injection site was approximately $\frac{1}{2}$ inch (1.3 cm) lateral to and 1 inch (2.6 cm) behind the umbilical stump. The needle was inserted at an angle of 45° to the skin surface and aimed at the lamb's rump. The effects of three doses of glucose were examined: 0.5 g/kg (10 ml/kg of a 5% solution), 1.0 g/kg (10 ml/kg of a 10% solution) and 2.0 g/kg (10 ml/kg of a 20% solution).

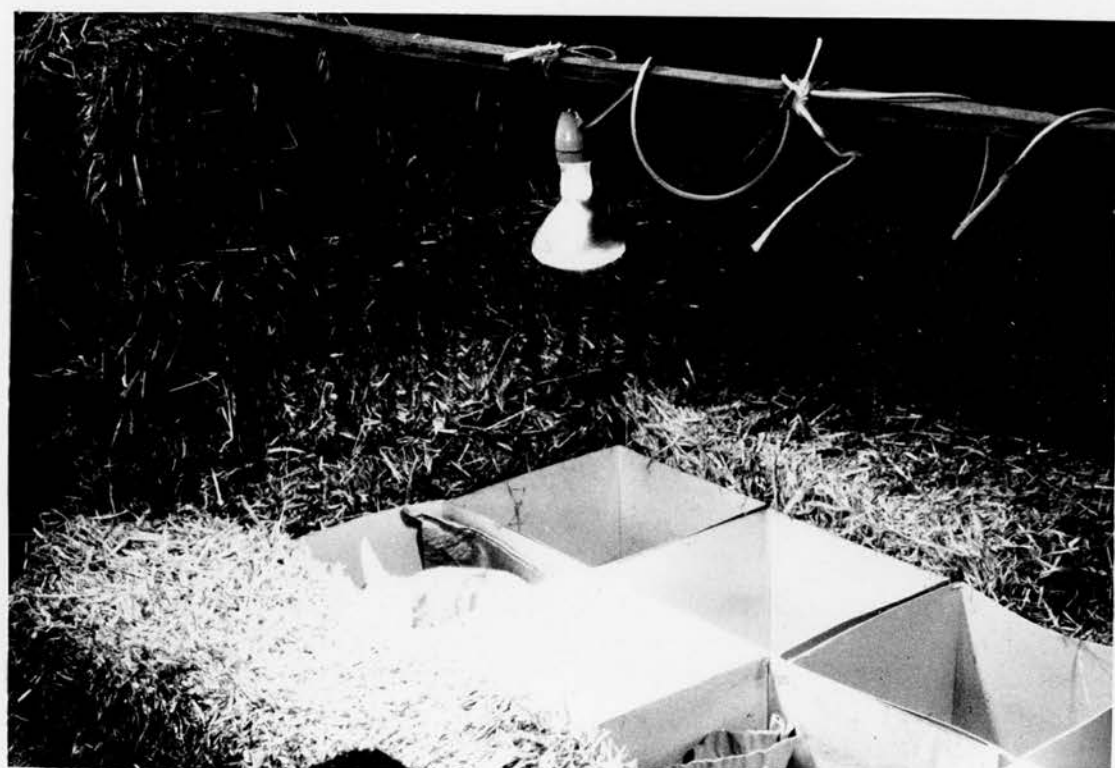
(4) Post-warming Care. In the initial stages of the project no special measures were employed to care for the resuscitated lambs. They were either returned to the ewe or placed in a 'lamb bar' where they received limited attention. This resulted in a high rate of mortality, especially in lambs allocated to the lamb bar, and so a post-resuscitation regime was progressively developed with the objective of improving survival. The main features of this regime were as follows:

- (i) After removal from the warmer the lambs were given 100-200 ml colostrum by stomach tube.
- (ii) Lambs were placed in a cardboard box measuring approximately 2ft x 2ft x 2ft (0.6 m). The box was bedded with newspaper and warmed by a 275 W infra-red lamp suspended four feet (1.3 m) above the lamb (Fig. 5.4). The box was destroyed after use.

Figure 5.3 Performing an intraperitoneal injection of glucose solution.



Figure 5.4 Unit used to house lambs after warming with individual cardboard box pens and an infrared lamp suspended four feet above the lamb.



- (iii) Lambs were fed three times daily on cow colostrum or milk substitute. A bottle was used if the lamb could suck vigorously, if not, it was fed by stomach tube at a dose rate of 50 ml/kg per feed.
 - (iv) Oral antibiotic, 1 ml containing 50 g ampicillin trihydrate and 100 mg activated attapulgate (Penbritin Oral doser; Beecham) was given to the lamb twice daily.
 - (v) Lambs were returned to the ewe as soon as they could stand and suck vigorously.
 - (vi) If for any reason the lamb could not be returned to the ewe, but was physically strong and able to suck, it was removed to a lamb bar after two days where care was taken to ensure that good nutrition continued. Any lamb which was weak or showing signs of disease was retained in the small pen until fit.
- (d) Monitoring of treatment. Lamb and warmer temperatures were monitored using electronic thermometers fitted with suitable thermister probes.

Heparinised blood samples were taken as previously described (Chapter 4) before warming, one hour after the injection of glucose solution and again on removal from the warmer. The packed cell volume and plasma glucose concentration were later estimated.

The progress of resuscitated lambs was followed until weaning. In order to investigate this without the complication of maternal effects ten resuscitated lambs together with nine healthy lambs which had not been hypothermic were artificially reared using a lamb bar. The lambs were weaned at a body weight of 15 kg. The health and growth rates of these two groups of lambs were monitored and compared.

Postmortem examinations were carried out on the 15 lambs previously described (Chapter 4).

5.2.2 Results

(a) Detection. The mean temperature of the hypothermic lambs was $29.6 \pm 0.52^{\circ}\text{C}$ ($n = 89$) with a range of $16.0 - 37.5^{\circ}\text{C}$. Only four lambs were detected in the temperature range of $37.0 - 39.0^{\circ}\text{C}$.

(b) Treatment. The effectiveness of the resuscitation regime is shown in Table 5.1. 73 lambs (82%) were successfully resuscitated. Sixteen lambs died during resuscitation. In four cases death was attributed to extreme hypothermia (rectal temperature less than 20°C). In nine cases death was attributed to hypoglycaemia. These lambs exhibited convulsions which could be temporarily arrested by the intravenous injection of 5 ml of a 20% glucose solution. One lamb was destroyed because of gross abdominal distension and the cause of failure in two lambs was unknown. The mean time for resuscitation was 2.6 ± 0.18 h ($n = 67$).

Of the 73 lambs which were successfully resuscitated, 48 (66%) were alive at weaning at three to four months of age. The causes of death in the remaining 25 lambs were diagnosed as follows: uncomplicated starvation (six), trauma (two), inhalational pneumonia following bottle feeding (five), infections (four), patent ductus arteriosus and/or incomplete expansion of the lungs (four) and causes unknown (four). In 22 of these lambs starvation was implicated as a contributory cause of death by an antemortem plasma glucose level below 1.5 mM and/or an absence of periodic acid-Schiff positive material in the liver indicating the exhaustion of hepatic glycogen reserves. The mean time between resuscitation and death was 6 ± 2 days ($n = 25$) but 18 of the 25 lambs died within three days of resuscitation. The survival rate after resuscitation to weaning improved as the work progressed and as the post-warming care was improved. Of the first

Table 5.1 Development phase - the effectiveness of treatment.

	Age at detection (hours)		All lambs
	6 or less	More than 6	
Lambs treated	40 (100%)	49 (100%)	89 (100%)
Lambs resuscitated	37 (93%)	36 (74%)	73 (82%)
Lambs alive at weaning (3-4 months)	30 (75%)	18 (37%)	48 (54%)

20 lambs successfully resuscitated only nine (45%) survived to weaning but 17 (85%) of the last 20 lambs survived.

Treatment appeared to be most effective both in terms of successful resuscitation and survival to weaning in the lambs aged less than six hours. Hypoglycaemia during rewarming and starvation afterwards appeared to be the major sources of loss in the older lambs.

The bale warmer performed well. On occasions maintenance of the correct temperature was sometimes a tedious procedure but the desired result was always achieved. On one of the farms the device was used to warm a calf which had become hypothermic after falling into a river.

The effects of the glucose injection on the plasma glucose concentration and packed cell volume are shown in Table 5.2. In the lambs aged less than six hours only the higher doses of glucose (1.0 and 2.0 g/kg) caused significant elevations of the plasma glucose level and there were no significant changes in packed cell volume. However, in the lambs aged more than six hours all doses of glucose caused a significant elevation of the plasma glucose level. The magnitude of the elevation was positively related to the dose of glucose. There were no significant changes in packed cell volume with the lower glucose doses but the highest dose rate (2.0 g/kg) did result in a small but significant elevation.

Good health was maintained in all of the 19 artificially reared lambs. There were no significant differences between the hypothermic lambs and the normal lambs in birth weight, body weight at eight weeks of age or the age at weaning. The mean value for birth weight was 2.8 ± 0.21 kg, for body weight at eight weeks of age 16.9 ± 0.75 kg and for age at weaning 48 ± 1.8 days.

Table 5.2 The effects of an intraperitoneal injection of glucose solution on the plasma glucose concentration and on packed cell volume in 64 hypothermic lambs (mean \pm SEM). Significant changes in the plasma glucose concentration are shown at the latter time.

Age of lamb (hours)	Glucose dose (g/kg)	Number of lambs	Plasma glucose concentration mM			Change in packed cell volume during first hour after injection (%)
			On presentation	One hour after injection	On removal from warmer	
0 - 6	0	6	2.2 \pm 0.53	2.2 \pm 0.48	2.3 \pm 0.48	-1 \pm 0.4
	0.5	21	3.9 \pm 0.65	4.4 \pm 0.70	3.7 \pm 1.78*	-1 \pm 0.6
	1.0 (2 lambs)	4	3.2 \pm 1.26	13.2 \pm 3.02*	10.2 \pm 3.94	0 \pm 1.0
	2.0 (2 lambs)					
> 6	0.5	15	0.5 \pm 0.11	1.6 \pm 0.30**	1.2 \pm 0.26	-1 \pm 0.8
	1.0	8	0.5 \pm 0.23	2.7 \pm 0.60***	2.5 \pm 1.00	0 \pm 0.7
	2.0	10	0.8 \pm 0.22	5.2 \pm 1.02***	6.6 \pm 1.35*	2 \pm 0.8*

5.2.3 The Final Treatment Regime

The outcome of the work just described was the definition of the resuscitation regime shown in Figure 5.5.

The early detection of hypothermia was clearly a desirable objective and thus the temperature of any lamb appearing weak should be taken at once.

Lambs with temperatures in the range of 37.0°C - 39.0°C were not considered to be in need of active rewarming and thus conservative treatment was advocated; drying of the coat and feeding.

The administration of glucose by injection to lambs aged less than five hours was considered unnecessary since hypoglycaemia was not a problem in these lambs. However, hypoglycaemia was a significant problem in the older lambs and only the highest dose of glucose used (2.0 g/kg) was effective in reversing hypoglycaemia in all lambs. This dose rate was associated with a small but significant elevation of the packed cell volume, presumably because water was drawn from the circulation into the peritoneal cavity. The maximum practical volume for injection is 50 ml and thus the maximum dilution which can be employed with a dose of 2 g/kg is 200 g/l . In view of the increase in packed cell volume which was observed when this solution was used, it seemed imprudent to employ a more concentrated solution.

Drying of the lamb before warming prevented further hypothermia in the initial stages of warming due to the evaporation of water from the coat.

Lambs were fed immediately after warming and nutrition was then assured to prevent starvation. The use of the stomach tube was advocated in all but the strongest lambs to avoid the risk of inhalational pneumonia associated with careless bottle feeding.

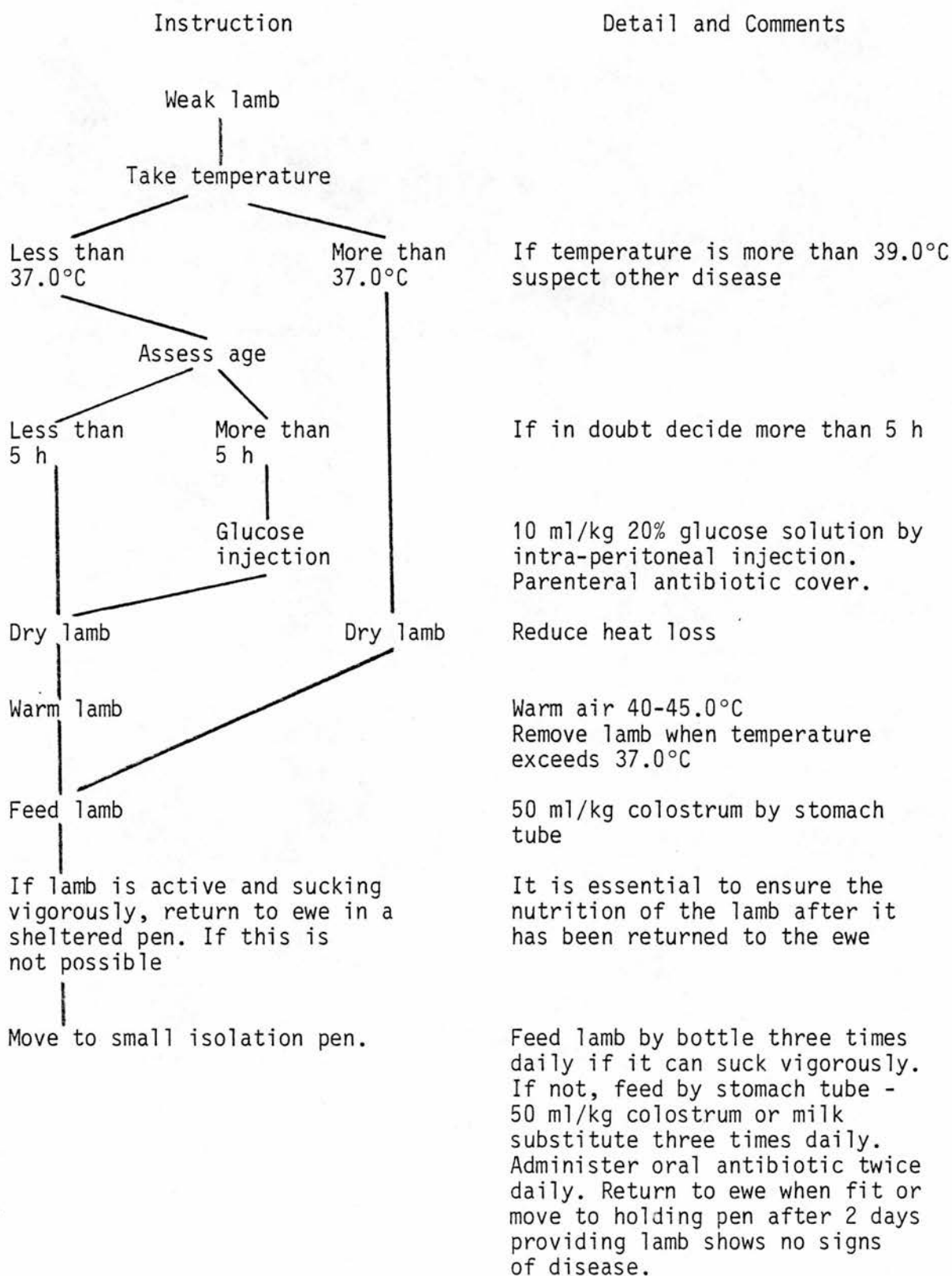


Figure 5.5 The Treatment of Hypothermic Lambs

The post-warming care regime, which was mainly aimed at lambs which were too weak to be returned to their ewes, was designed to ensure nutrition, to prevent further hypothermia and to prevent infectious disease such as enteritis.

5.3 FIELD TRIAL

5.3.1 Methods

- (a) Plan. This trial was conducted on the 30 farms mentioned in Chapter 4. On a visit to each farm before lambing, the staff were equipped with a thermometer (see below) and were instructed in the techniques outlined in Figure 5.5. Each farm was subsequently visited during lambing to review progress.
- (b) Detection of hypothermia. Each farm was supplied with an electronic thermometer designed and built by Mr M.G. Christie of the Moredun Research Institute (Fig. 5.6). By means of flashing coloured lights this device indicated the rectal temperature of the lamb to be either 39.0°C or greater (green, amber and red lights), $37.0 - 39.0^{\circ}\text{C}$ (amber and red lights) or less than 37.0°C (red light). These temperature ranges were chosen to aid the selection of treatment required according to Figure 5.5. The thermometer was based on a thermistor sensor located in the tip of the rectal probe and on 'C - MOS' circuits chosen for their very low power consumption and lack of dependence on a constant voltage supply.
- (c) Treatment of hypothermia. The treatment regime used was basically that indicated in Figure 5.5. The farm staff were encouraged to take the temperature of any lamb that appeared at all weak and then to act accordingly. For the sake of simplicity the dose of glucose solution

Figure 5.6 The electronic thermometer designed and constructed
by Mr M.G. Christie.



for injection (200 g/l) was approximated to:

Large lamb (more than 4.5 kg)	50 ml
Medium lamb (3.0 - 4.5 kg)	35 ml
Small lamb (less than 3.0 kg)	25 ml

The administration of long acting parenteral antibiotic at the same time as the injection of glucose solution was included to protect the lamb from any infection introduced during the intra-peritoneal injection.

Two types of warmer were used in the trial. On 20 farms the bale warmer, already described, was used. On ten of the farms a smaller warming box was used (Fig. 5.7). This box was approximately 1.2 m long, 0.5 m wide and 0.5 m high and was separated vertically by a sheet of weld mesh into two chambers, one 0.5 m long and the other 0.7 m. A two kilowatt fan heater was placed in the smaller chamber and the lamb was placed in the larger chamber on a raised wire mesh floor.

The dose rate of colostrum for feeding (50 ml/kg) was simplified to large lamb - 200 ml; medium lamb - 150 ml and small lamb - 100 ml.

(d) Recording. The farmers were asked to record the progress of the lambs in the same questionnaire mentioned in Chapter 4. The additional information requested was: success of resuscitation (alive or dead on removal from the warmer), survival for one week after resuscitation (yes or no) and the fate of the lamb (returned to a ewe or artificially reared).

5.3.2 Results

The distribution of the lambs according to age and temperature on detection is shown in Table 5.3, together with the effects of treatment. The fate of lambs which survived for more than one week after

Figure 5.7 The small wooden lamb warming box used on ten of the farms in the field trial.



Table 5.3 Field trial - the effectiveness of treatment according to age and temperature on detection.

Lamb age (hours)	5 or less			More than 5			All lambs
	Lamb temperature °C	37.0-39.0	<37.0	All lambs	37.0-39.0	<37.0	All lambs
Lambs treated		63 (100%)	164 (100%)	227 (100%)	72 (100%)	199 (100%)	271 (100%)
Lambs resuscitated		61 (97%)+	142 (87%)	203 (89%)	65 (90%)+	163 (82%)	228 (84%)
Lambs alive one week later		56 (89%)	117 (71%)	173 (76%)	56 (78%)	117 (59%)	173 (64%)

+ Immediate condition improved

resuscitation is shown in Table 5.4.

Hypothermia was detected in the early stages of the condition (rectal temperature of 37.0 - 39.0°C) in 135 cases (27%) and this early detection was associated with highest survival rate to one week. Age at detection also seemed to have an influence on the success rate with the poorest results recorded in the older lambs. There was considerable farm to farm variation in the success of treatment. Considering only farms which treated more than 20 lambs ($n = 8$) the range for successful resuscitation was 76 - 97% and for survival for one week 53 - 89%. Only 8% of the lambs which survived for at least one week were eventually artificially reared, the majority being returned to ewes. Most of the lambs which were artificially reared were twins and triplets.

In general, the detection and treatment procedures were well received. The electronic thermometer, however, met with a mixed response. Many farmers were delighted with the instrument whilst others were not convinced of its usefulness. The glucose injection naturally caused some apprehension but this was generally resolved once the farmers had been shown the correct technique. Of the 67 lambs not successfully resuscitated, 33 were submitted to other agencies for postmortem examination. In three cases only was death attributed to poor injection technique. Few problems were encountered in the use of the bale warmer. A low air temperature was a problem when wet bales were used. Superior results were reported when hay bales were used instead of straw probably because they are more rectangular in shape, making the finished warmer more air-tight. The most serious criticisms of this warmer were related to its bulk and lack of portability. Overheating was a serious problem in the small

Table 5.4 Field trial - fate of lambs which survived at least one week after resuscitation.

Type	Single	Twin	Triplet	All lambs
Returned to a ewe	31 (97%)	205 (93%)	75 (87%)	311 (92%)
Artificially reared	1 (3%)	16 (7%)	11 (13%)	28 (8%)

wooden warmer. The thermometers used to monitor the temperature in these heaters had a maximum calibration point of 55.0°C and many thermometers were burst. The problem was overcome by removing the back of the smaller chamber and placing the fan heater outside the box. This problem was likely related to the small volume of this box (0.3 m³) when compared with that of the bale warmer (1.5 m³).

Care of the lambs after warming varied considerably from farm to farm. In many cases it was clear that inadequate attention was being paid to the nutrition of these lambs.

5.4 DISCUSSION

The techniques finally developed were remarkably effective. All concerned with the field trial commented that the results were considerably better than they would have expected using former methods. When considering these results it should be remembered that they include lambs suffering such severe hypothermia that death was inevitably imminent, small immature lambs in which a high rate of mortality would be normally expected and lambs suffering other disease which may well have not been detected and specifically treated. The satisfactory progress of the artificially reared 'ex-hypothermic' lambs in the development part of this work and the successful return of the majority of lambs to ewes in the field trial demonstrate that the treatment of hypothermia is a commercially worthwhile occupation. The results observed in the field trial were better than those recorded in the development phase but only marginally so. This could suggest that the improvements in treatment which were only applied towards the end of the development phase but were applied throughout the field trial had little or no effect. However, lambs treated in the development phase were under

constant veterinary supervision whereas lambs in the field trial were treated by staff with no veterinary training and practically no supervision. A more reasonable conclusion is that the improvements in treatment compensated for the inexperience of the operators.

Early detection of hypothermia increased the chances of successful treatment. In the development phase the shepherds did not use thermometers and only 4% of lambs were detected in the temperature range of 37.0 - 39.0°C. In the field trial where thermometers were used (by some) the corresponding figure was 27%. This was clearly an improvement but still 73% of the lambs were not detected until rectal temperature had fallen below 37.0°C. On the farms lambing outside, this was probably unavoidable in many cases since the lambs were not under constant supervision, but on the farms lambing inside the majority of cases should have been detected in the early stages. As described earlier, lambs in the early stages of hypothermia can appear just 'a little weak' and this appearance was apparently not recognised as a signal for immediate attention. The early detection of hypothermia, for which a thermometer is essential, improves the chances of the lamb's survival and also saves time since the treatment is not complicated and the lamb need not necessarily be removed from its ewe.

In both the development phase and the field trial the poorest results were obtained in the older lambs in which the major cause of hypothermia was starvation. In the development phase hypoglycaemia during warming and starvation afterwards probably accounted for this but in the field trial both these problems should have been largely avoided. Two factors probably account for the lower success rates in these lambs. First, they were more likely to be suffering other disease such as enteritis which does not affect lambs as young as five hours,

and second, the combined problems of hypothermia and starvation are a more severe insult to a lamb than is hypothermia alone.

The bale warmer was bulky and it was sometimes difficult to maintain the correct temperature. It was also evident that the use of an electric heater in close proximity to straw bales and the inevitable loose straw might prove a fire risk. In spite of these problems which were later overcome (see Section 5.5) the device proved safe and effective. The small wooden box warmer did not. Overheating was a common problem which led to hyperthermia in the lambs on a few occasions. The immediate remedy that was applied cannot be regarded as a satisfactory solution of the problem and this device cannot be recommended. The problem was almost certainly related to the low volume of the box (only 20% of that of the bale warmer).

The intra-peritoneal injection of glucose solution in lambs likely to be hypoglycaemic proved an effective and practical technique. The few problems that did occur due either to faulty injection technique or the use of non-sterile equipment and solutions, serve to demonstrate the need for proper instruction before this technique is practised.

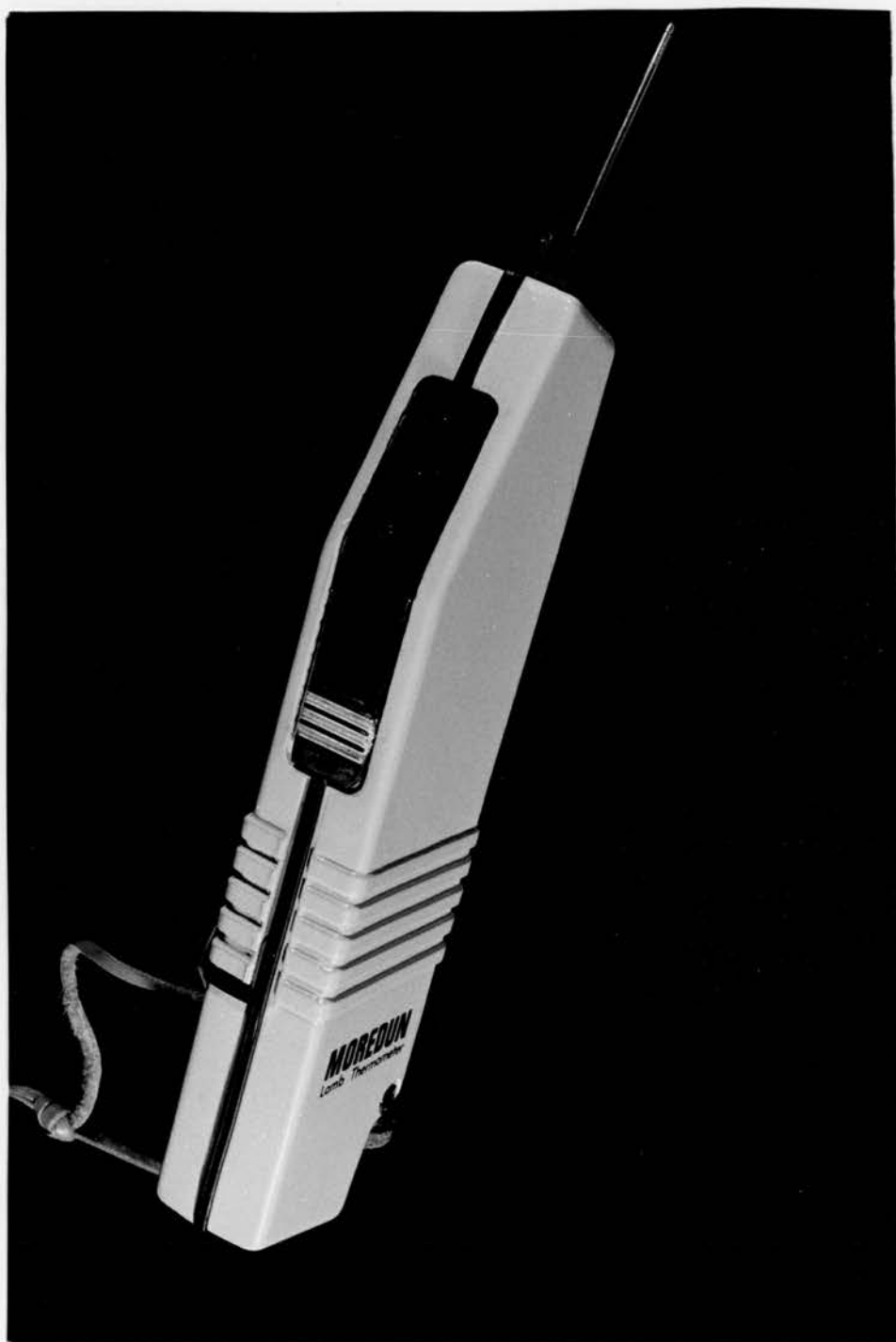
The standards of post-warming care on some of the farms in the field trial left much to be desired. This aspect of treatment is less dramatic than the resuscitation procedures but the results from the development phase demonstrate that it is just as important.

5.5 FURTHER DEVELOPMENTS

Since the completion of the work described above the techniques and equipment have been developed and widely adopted.

A 'Hypothermia Indicator' based on the design of the electronic thermometer used in the field trial is now commercially available (Fig. 5.8; Macam Ltd, Livingston, Scotland).

Figure 5.8 A Moredun Lamb Thermometer (Macam Ltd, Livingston,
Edinburgh).

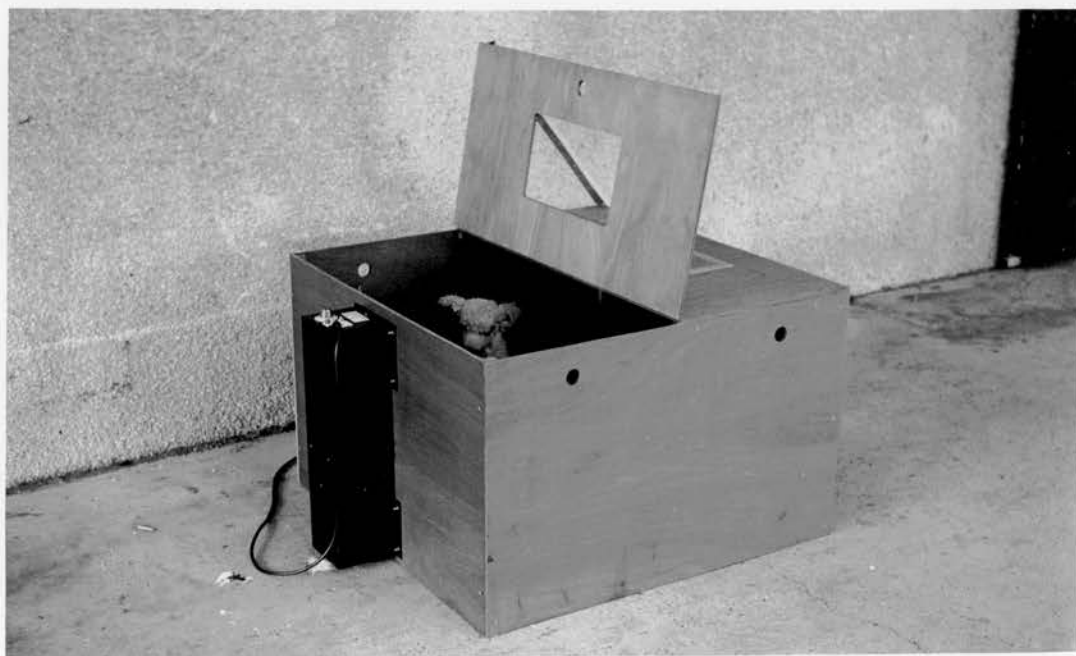


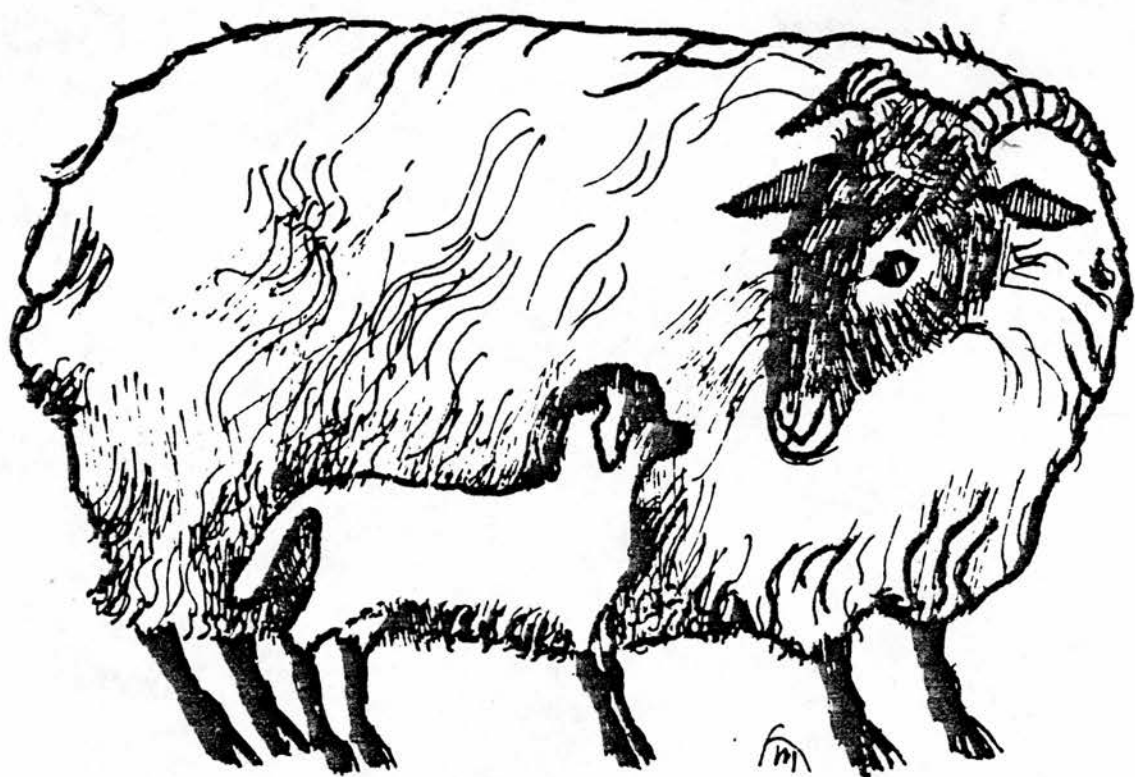
The warming box underwent a number of developments. The problems of bulk, portability, fire risk and, to some extent, temperature control, were overcome by constructing a box in wood to the internal dimensions of the bale warmer (Fig. 5.9). Adjustable ventilators were fitted in the sides to permit temperature control. This device proved easier to use than the bale warmer but temperature control was still a problem especially for the occasional user. All problems were overcome in the 'Moredun Lamb Warming Box' (Fig. 5.10; Macam Ltd, Livingston, Scotland). In this device air is circulated around the box by a fan and controlled heating is provided by an electric heater fitted with a thermostat. Ventilation with fresh air is provided by holes in the bottom and top of the sides.

Measures for both the prevention and treatment of hypothermia in newborn lambs based to a considerable extent on the work described in this thesis are now advocated to farmers by the Agricultural Training Board, the Scottish Agricultural Colleges and the Agricultural Development and Advisory Service.

Figure 5.9 A wooden lamb warming box constructed to the internal dimensions of the bale warmer showing the adjustable ventilations.

Figure 5.10 A 'Moredun lamb warming box' (Macam Ltd, Livingston, Edinburgh).





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APPENDIX

Published Work

The following papers have been published. A reprint of each is appended.

Eales, F.A. (1982). Detection and treatment of hypothermia in newborn lambs. In Practice (Supplement to the Veterinary Record) 4, 20-22.

Eales, F.A., Dickson, I.A., Lloyd, M. and Smith, M.E. (1983). Hypothermia in the young lamb. Technical Note No. 60. The Scottish Agricultural Colleges.

Eales, F.A., Gilmour, J.S., Barlow, R.M. and Small, J. (1982). Causes of hypothermia in 89 lambs. Veterinary Record, 110, 118-120.

Eales, F.A. and Small, J. (1980). Determinants of heat production in newborn lambs. International Journal of Biometeorology, 24, 157-166.

Eales, F.A. and Small, J. (1980). Summit metabolism in newborn lambs. Research in Veterinary Science, 29, 211-218.

Eales, F.A. and Small, J. (1981). Effects of colostrum on summit metabolic rate in Scottish Blackface lambs at five hours old. Research in Veterinary Science, 30, 266-269.

Eales, F.A., Small, J. and Armstrong, R.H. (1980). Plasma composition in hypothermic lambs. Veterinary Record, 106, 310.

Eales, F.A., Small, J. and Gilmour, J.S. (1982). Resuscitation of hypothermic lambs. Veterinary Record, 110, 121-123.

Eales, F.A., Small, J., Oliver, J.S. and Quigley, C. (1981). Phenol poisoning in a newborn lamb. Veterinary Record, 108, 421.

In addition to the above, the following papers have also been published during the period of this work:

- Eales, F.A. (1980). Hypothermia in newborn lambs. Advisory leaflet. Animal Diseases Research Association.
- Eales, F.A. (1980). The detection and treatment of hypothermia in newborn lambs. Advisory booklet. Animal Diseases Research Association.
- Eales, F.A. (1981). Hypothermia in newborn lambs; causes, prevention and treatment. The Blackface Sheep Breeders Association Journal, 33, 21-22.
- Eales, F.A. (1981). Hypothermia in newborn lambs; causes, prevention and treatment. Training course manual with slides. Agricultural Training Board.
- Eales, F.A. (1982). Some aspects of general anaesthesia in the newborn lamb. Proceedings of the Association of Veterinary Anaesthetists of Great Britain and Ireland, 10, 208-215.
- Eales, F.A. (1983). Hypothermia. The Sheep Farmer, 2, No. 3, 11-13.
- Eales, F.A. (1983). Hypothermia in newborn lambs. In "Diseases of Sheep", ed. W.B. Martin. Oxford, Blackwell Scientific Publications, In press.
- Eales, F.A. (1983). Hypothermia in newborn lambs. Educational tape with booklet. Unit for Veterinary Continuing Education. The Royal Veterinary College. In press.
- Eales, F.A., Murray, L. and Small, J. (1982). Effects of feeding ewe colostrum, cow colostrum or ewe milk replacer on plasma glucose in newborn lambs. Veterinary Record, 111, 451-453.
- Eales, F.A. and Small, J. (1982). Alphaxalone/alphadolone anaesthesia in the lamb. Veterinary Record, 110, 273-275.
- Eales, F.A., Small, J. and Gilmour, J.S. (1983). Neonatal mortality of lambs and its causes. In "Sheep Production", ed. W. Haresign. pp.289-298. London, Butterworth Scientific Ltd.

Summit metabolism in newborn lambs

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Basal metabolic rate and then summit metabolic rate were measured using a water immersion technique in newborn lambs at 1 h or 4 h old. The mean rates of basal and summit metabolism were 51 ± 1.5 and 235 ± 7.9 W per m^2 respectively. Summit metabolic rate was more closely related to body surface area than to body weight. Umbilical catheters were inserted at birth and blood samples were taken at 5 min of age and during the measurement of metabolic rate to examine changes in acid-base status and energy substrate concentrations. At birth varying degrees of metabolic acidosis and hyperlactaemia were observed. Summit metabolism was associated with the development of a further metabolic acidosis and with increases in the blood levels of lactate and excess lactate and the plasma levels of glucose, glycerol and free fatty acids. The relationships between summit metabolic rate, the metabolic state at birth and the change in metabolic state caused by cooling suggested that prepartum hypoxia was associated with a depression of the thermogenic response to cold stress and also with a depression of sympathetic nervous activity.

HYPOTHERMIA IS A major cause of death in the new born lamb (Alexander 1962; Gunn and Robinson 1963) and occurs when the rate of heat loss exceeds the rate of heat production. Alexander (1962) identified considerable variation in the maximum rate of heat production (summit metabolism) among Australian lambs but further extensive studies failed to identify the source of this variation (Alexander *et al* 1968; Alexander 1970; Alexander and Williams 1970; Alexander *et al* 1972; Alexander and Bell 1975a, b).

The present study was conducted to establish whether similar variation in heat production capacity existed between British lambs and, if it did, to investigate the possibility that this variation might be related to the metabolic state of the lamb at birth. To these ends 'basal' metabolic rate (the rate of metabolism recorded at rest under thermoneutral conditions) and 'summit' metabolic rate (the rate of metabolism during a decline of rectal temperature from 39° to 37°C induced by cold stress) were measured in lambs using a water immersion tech-

nique. Water was used as a cooling medium in preference to air as used by Alexander (1961) because the apparatus had a simple construction, problems of obtaining blood samples during cold stress in sub-zero temperatures were avoided and the lambs were continually accessible. Blood samples were taken through indwelling umbilical cannulae from most of the lambs at birth and during basal and summit metabolism in order to monitor acid-base status and energy substrate concentrations.

Materials and methods

Animals

Pregnant Scottish Blackface ewes were brought indoors four weeks before the anticipated lambing date. For three weeks the ewes were maintained in a covered yard and then they were removed to individual pens. The ewes were freely fed a proprietary complete diet (Ruminant A, Seaford Mill). Lambs were born at ambient temperatures of 13° to 17°C , were left with the ewes for 30 min but were prevented from sucking by human interference. The mean weight of the lambs was 3.95 ± 0.15 kg ($n=29$).

Water immersion and cooling procedures

A face mask was secured to the lamb with a tape, and a thermistor probe was inserted into its rectum. The lamb, supported by a foam lined platform, was then placed in a water tank at 38.5°C (Fig 1). The tank was 550 mm deep, 500 mm long and 260 mm wide. Water was introduced just above the tank base at a controlled rate and temperature and circulated by six pumps. In every case the water temperature was maintained at 38.5°C (thermoneutral) for 20 min after a steady metabolic rate ('basal' metabolic rate) had been achieved. Cold stress was induced after thermoneutral immersion by cooling the water at a rate of 0.4 to 0.5°C per min. The water temperature was decreased at this rate until the rectal temperature had fallen, after an initial rise, to the lowest value recorded during thermoneutral immersion (Fig 2).

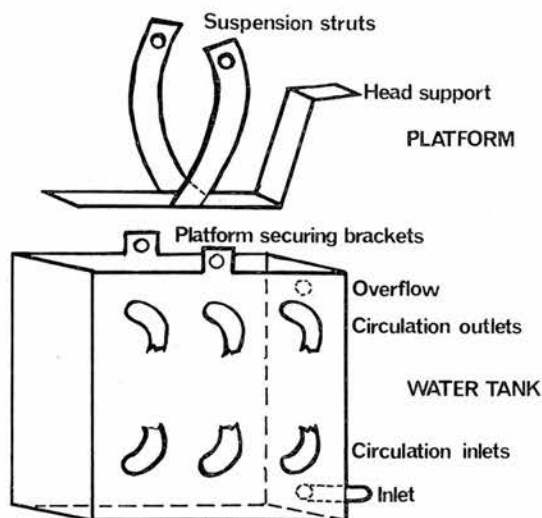


FIG 1a: The water tank and lamb supporting platform. The circulation ports on the far side of the tank have been omitted for the sake of clarity

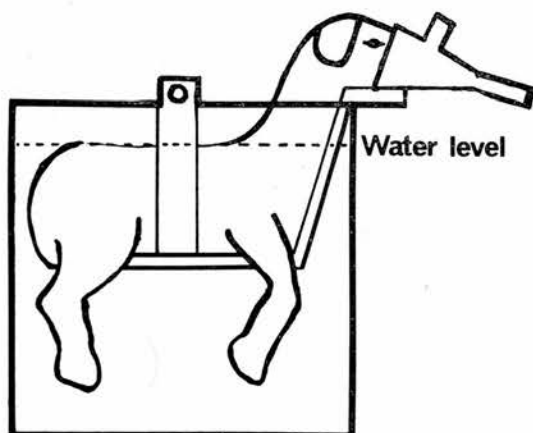


FIG 1b: A lamb in the water tank showing the supporting platform and face mask in place

Water cooling was then stopped. When rectal temperature had fallen to 37°C the lamb was removed from the water and dried. The water temperature at which water cooling was stopped varied from lamb to lamb with a range of 14° to 28°C.

Metabolic rate

The oxygen concentration in the mixed outlet air from the face mask was estimated with a paramagnetic analyser (type OA 137, Taylor-Servomex)

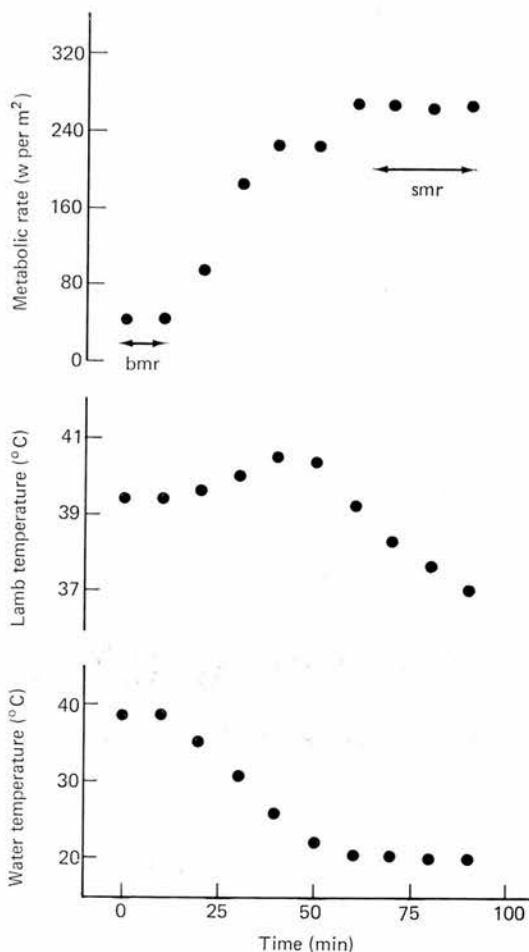


FIG 2: Metabolic rate, rectal temperature and water tank temperature during the measurement of basal metabolic rate (bmr) and summit metabolic rate (smr) in a 4 h old male Blackface singleton lamb of body weight 4.60 kg

and the carbon dioxide concentration with an infra-red analyser (Model 2, Sir Howard Grubb Parsons) (Fig 3). Mass air flow, regulated to maintain a carbon dioxide concentration of below 1 per cent in mixed outlet air was estimated with a pressure drop transducer (Model EA11-50KX, Teledyne Hastings Raydist). The calibrations of gas analysers were regularly authenticated by the use of previously calibrated gas mixtures. The calibration of the pressure drop transducer was checked by coupling with a wet gas meter (type DM3E, Alexander Wright).

Metabolic rate was calculated from oxygen consumption and carbon dioxide production according to Brouwer (1965) and was expressed per unit

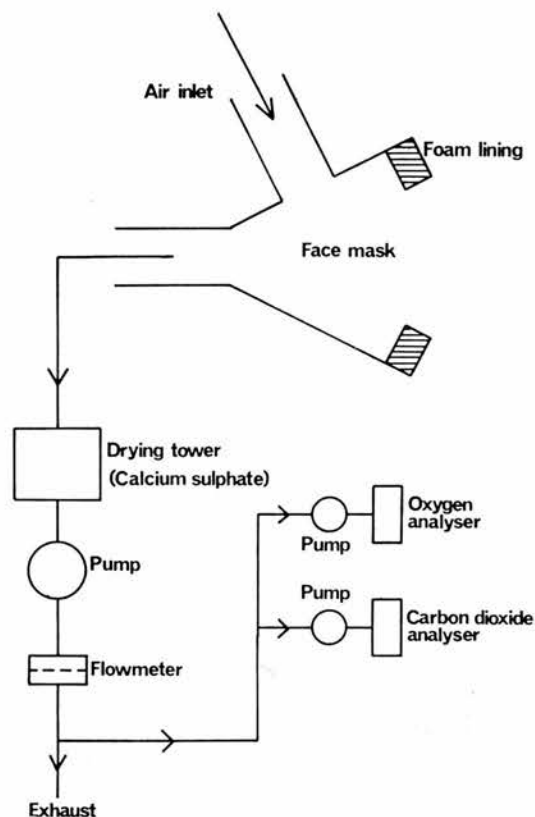


FIG 3: A cross-section of the face mask and a scheme of the open circuit indirect calorimetry system

body weight and per unit body surface area, the latter derived according to Peirce (1934). The average rate of metabolism during the last 10 min of thermoneutral immersion was taken as a measure of basal metabolic rate. The average rate during the fall of rectal temperature from 39° to 37°C, within which range metabolic rate is not body temperature dependent (Alexander 1962), was taken as a measure of summit metabolic rate.

Basal metabolic rate was estimated in 39 lambs: 13 Blackface and five Blackface cross (Border Leicester sire) lambs at 1 h old and 18 Blackface and three Blackface cross lambs at 4 h old. Summit metabolic rate was estimated in 29 of these lambs: eight Blackface and five Blackface cross lambs at 1 h of age and 13 Blackface and three Blackface cross lambs at 4 h of age. The remaining 10 lambs (five Blackface lambs aged 1 h and five Blackface lambs aged 4 h) were not exposed to cold stress after the initial period of thermoneutral immersion but were maintained at a water temperature of 38.5°C for a

further 70 min in order to investigate the effects of prolonged thermoneutral immersion.

Blood sampling and analysis

At birth a catheter was inserted into an umbilical vein of 24 lambs and 5 min after birth a 5 ml sample of blood was taken into a heparinised syringe. A further sample was taken at the beginning of the measurement of basal metabolic rate. Nineteen lambs subjected to cold stress (11 Blackface and one Blackface cross lambs at 1 h and seven Blackface lambs at 4 h old) were then sampled at the end of the measurement of basal metabolism and at 5 min intervals during summit metabolism, while the five Blackface lambs subjected to prolonged thermoneutral immersion at 1 h old were further sampled at 20 min intervals. The maximum blood volume taken was 50 ml.

Samples for the estimation of blood lactate and pyruvate levels were treated and the levels estimated according to the methods of Hohorst (1963) and Bucher *et al* (1963) respectively. The blood was immediately deproteinised after sampling with ice cold perchloric acid and a supernatant recovered by refrigerated centrifugation. The supernatant was then stored for up to 24 h at -40°C until analysis. Blood haemoglobin was estimated by the cyanmethaemoglobin method. Blood pH and the partial pressure of carbon dioxide were estimated using a micro pH electrode and a Severinghaus carbon dioxide electrode (IL Laboratories). If immediate pH and gas estimations were not possible samples were stored for up to 2 h in iced water. Plasma was stored at -20°C until analysis for glucose, free fatty acids and glycerol levels according to the methods of Trinder (1969), Lauwery (1969) and Eggstein and Kreutz (1966) respectively. Blood base excess was derived according to Siggaard-Anderson (1963) and excess lactate according to Huckabee (1958). The results were treated statistically by regression analysis and conventional *t* tests and are presented as mean \pm standard error of the mean (SEM) or as correlation coefficients.

Results

Behaviour

On immersion at 38.5°C lambs were restless for a few minutes but soon settled into a sleep-like state which was maintained until cooling when paddling movements and bleating were noticed. During summit metabolism paddling movements ceased, bleating was uncommon and the lambs shivered. When returned to the ewe the lambs were physically active responding to the ewes' attention by teat

seeking and sucking. Lambs subsequently developed in a similar manner to that observed in lambs not subjected to experimentation.

Metabolic rate and rectal temperature

Metabolic rate, lamb rectal temperature and water temperature during a typical experiment are shown in Fig 2. Prolonged thermoneutral immersion of 10 lambs caused no significant changes in metabolic rate or rectal temperature. Mean basal metabolic rate was 3.59 ± 0.10 w per kg or 51 ± 1.5 per m^2 ($n=39$), 10.6 ± 0.31 ml oxygen consumed per min per kg). Mean rectal temperature during basal metabolism was $39.4 \pm 0.05^\circ C$ ($n=29$). It rose to $40.1 \pm 0.08^\circ C$ in the initial stages of cooling and then decreased progressively. The mean time for rectal temperature to fall from 39° to $37^\circ C$ was 17 ± 3.2 min ($n=29$) and during this period metabolic rate remained relatively constant (Fig 2). Lamb breed and sex, and the age at cooling had no significant effect on the rate of summit metabolism and therefore all of the data were pooled. The mean summit metabolic rate was 16.5 ± 0.62 w per kg or 235 ± 7.9 w per m^2 ($n=29$), 47.6 ± 1.83 ml oxygen consumed per min per kg).

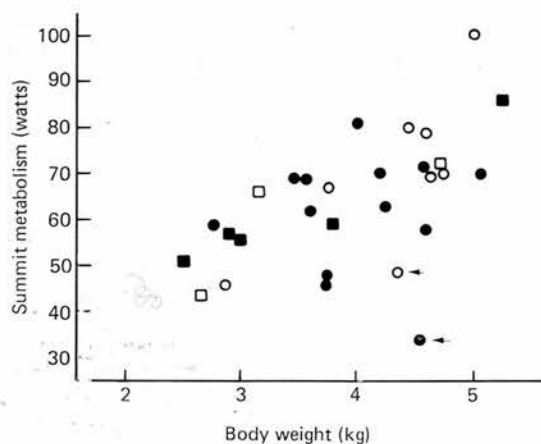


FIG 4: Relation of summit metabolic rate to body weight in 29 lambs aged 1 to 4 h. The two lambs marked with arrows showed low summit metabolic rates and atypical responses to cold stress and were omitted from analysis of these data. The round symbols represent Scottish Blackface lambs and the square symbols Scottish Blackface cross lambs. A closed symbol indicates the measurement of summit metabolic rate at 1 h of age and an open symbol measurement at 4 h

The relationship between summit metabolic rate and body weight is shown in Fig 4. Two lambs (indicated in Fig 4) were excluded from analysis

of these data because they showed uncharacteristic behaviour during cold stress. Paddling movements, bleating and shivering were absent and low summit metabolic rates were recorded. The relationship between summit metabolic rate and body weight was best described by the regression equation:

$$\text{Log}_e \text{ SM} = 3.28 + 0.65 (\log_e W)$$

where SM = summit metabolic rate (watts) and

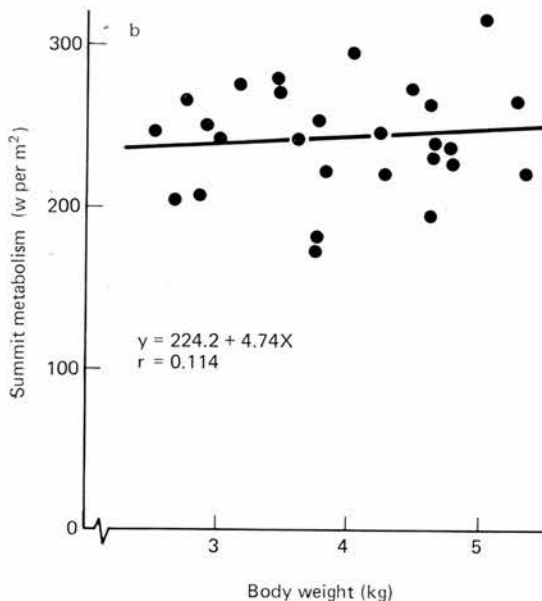
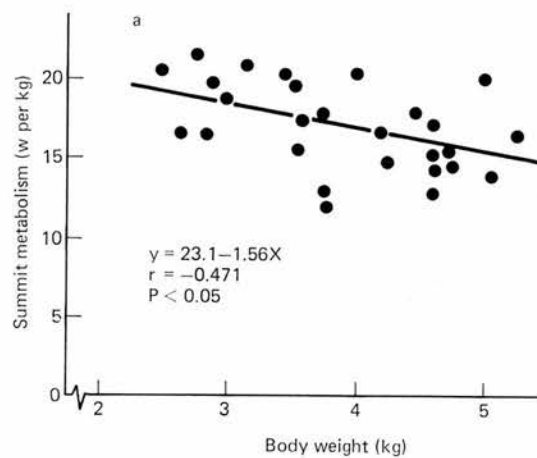


FIG 5: (a) Relation of summit metabolic rate per unit body weight to body weight in 27 lambs aged 1 to 4 h. (b) Relation of summit metabolic rate per unit body surface area to body weight in 27 lambs aged 1 to 4 h. Surface area was derived according to Peirce (1934); surface area (m^2) = $0.121 \times \text{body weight (kg)}^{0.59}$

W = body weight (kg). This equation may be written:

$$SM = 26.6 W^{0.65}$$

Body surface area is proportional to body weight raised to the power 0.59 (Peirce 1934). The exponent 0.65 (SE 0.132) in the above equation is significantly different from 1.00 ($P < 0.05$) but not from 0.59, so that summit metabolic rate was apparently more closely related to body surface area than to body weight. The relationships between body weight and summit metabolic rate per unit body weight and per unit body surface area are shown in Fig 5. Summit metabolic rate per unit body surface area was independent of body weight whereas summit metabolic rate per unit body weight decreased as body weight increased.

Acid-base status and energy substrate levels

Five minutes after birth all lambs exhibited high blood hydrogen ion levels and low base excess values indicative of a metabolic acidosis, ie, an acidosis of non-respiratory origin (Table 1). All lambs also exhibited a hyperlactaemia at this time and blood lactate concentration was negatively correlated with the base excess value ($r = -0.863$, $P < 0.01$) and positively with the plasma glucose concentration ($r = 0.772$, $P < 0.001$).

The changes in acid-base balance and energy

substrate levels caused by cooling are shown in Table 1. Cooling was associated with the development of a metabolic acidosis and hyperlactaemia, and increases in the plasma levels of glucose, glycerol and free acid fatty acids. The coefficients of correlation between the increments in metabolic rate from basal to summit metabolism and the associated changes in metabolite concentrations demonstrate that the magnitude of the increment in metabolic rate was positively correlated with the degree of metabolic acidosis and hyperlactaemia induced and with the rises in the plasma levels of glucose, glycerol and free fatty acids.

Regression coefficients relating summit metabolic rate to body surface area and to blood or plasma metabolite concentrations at birth are shown in Table 2. Body surface area alone accounted for only 13 per cent of the variation in summit metabolic rate whereas inclusion of blood lactate, base excess or hydrogen ions increased the accountability to between 40 and 50 per cent.

During prolonged thermoneutral immersion of five Blackface lambs at 1 h of age there was a significant reduction in plasma glycerol concentration from 229 ± 28.7 to 82 ± 21.9 $\mu\text{mol per litre}$ ($P < 0.01$). There were no significant changes in the other metabolite concentrations and mean values for the whole period of immersion are presented in Table 1. There were no significant differences between

TABLE 1: Blood composition in five lambs subjected to prolonged thermoneutral immersion and in 19 lambs subjected to cooling

	Prolonged thermoneutral immersion (n = 5) mean value (SEM) for the 80 min period	Birth mean value (SEM)	Lambs subjected to cooling (n = 19)		Correlations between the increment in metabolic rate from basal to summit levels and the corresponding changes in blood and plasma metabolite values (r)
			Basal metabolism mean value (SEM)	Summit metabolism mean value (SEM)	
Blood base excess m equiv per litre	3.2 (1.88)	-2.3 (1.75)	3.4 (1.53)***	-3.5 (0.99)***	-0.861***
Blood hydrogen ions g ion per litre 10^{-8}	4.56 (0.144)	6.26 (0.589)	4.58 (0.225)***	6.10 (0.164)***	0.719***
Blood lactate m mol per litre	3.86 (1.325)	5.82 (0.943)	3.91 (0.655)***	5.78 (0.456)***	0.626***
Blood excess lactate m mol per litre	-1.22 (0.611)	—	0	1.8 (0.38)***	0.729***
Plasma glucose m mol per litre	1.77 (0.277)	3.3 (0.36)	2.6 (0.34)*	5.4 (0.30)***	0.467*
Plasma glycerol $\mu\text{mol per litre}$	135 (21.6)	117 (21.9)	150 (35.4)	509 (50.5)***	0.606**
Plasma free fatty acids m mol per litre	0.45 (0.101)	0.41 (0.076)	0.38 (0.029)	1.36 (0.082)***	0.454*

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

In the cooled group the significance of the changes from birth to basal metabolism and from basal to summit metabolism are shown at the latter period. The right hand column shows correlations between the increment in metabolic rate from basal to summit levels and the corresponding changes in blood and plasma metabolite levels

TABLE 2: The effect of body surface area and of blood or plasma composition at birth on summit metabolism measured at either 1 or 4 h old in 19 lambs

Metabolite	a	b	c	Percentage of variation accounted for by coefficients
	Constant	Body surface area	Metabolite concentration	
None	-3.05	239.7		13
Base excess m equiv per litre	-18.15	302.9	1.09	40
Blood hydrogen ions g ion per litre 10^{-8}	2.12	304.7	-3.71	50
Blood lactate m M	-27.33	377.6	-2.41	49
Plasma glucose m M	-27.52	379.7	-4.31	23
Plasma glycerol m M	1.10	216.3	19.91	9
Plasma free fatty acids per m equiv per litre	-14.11	260.7	12.76	16

The regression equations are of the form $y = a + bx_1 + cx_2$ where y represents summit metabolism (w), x_1 body surface area (m^2) and x_2 the concentration of the metabolite

any of these values and those recorded during basal metabolism in lambs later subjected to cooling.

Discussion

Metabolic rate

Any increase in metabolic rate in the present study could have been related to the stress of immersion and indirect calorimetry, the thermogenic stimulus of cooling or to both these factors. Values for blood pH and base excess and the plasma concentrations of free fatty acids, glucose and glycerol during basal metabolism were similar to those obtained by other workers (Alexander and Mills 1968; Alexander *et al* 1972), and during prolonged thermoneutral immersion there were no changes in these values except for a decrease in plasma glycerol levels. These factors plus the behaviour of the lambs during thermoneutral immersion suggest that the metabolic rate recorded at this time was a reliable estimation of basal metabolic rate and that the increases in metabolic rate associated with cooling were due primarily to this stimulus.

The mean basal rate of oxygen consumption found in the present study of 10.6 ± 0.31 ml per min per kg ($n=39$) was significantly higher ($P < 0.01$) than the value of 9.4 ± 0.22 ml per min per kg ($n=29$) recorded by Andrews *et al* (1973) in crossbred lambs aged 1 to 6 h. However these workers used air to obtain a thermoneutral environment and this factor together with the different breed and age of the lambs studied hinder interpretation of this difference in basal metabolic rate.

The mean value for summit metabolic rate of

235 ± 7.9 w per m^2 ($n=29$) is significantly lower ($P < 0.05$) than the value of 261 ± 8.5 w per m^2 ($n=19$) observed by Alexander (1962) in merino lambs aged less than 9 h. Lambs with very low heat production capabilities may have been excluded from Alexander's study since it is likely that they would have died or become moribund soon after birth before summit metabolic rate could have been estimated. If the two lambs showing an atypical and low response to cooling are removed from our data, the corrected mean rate of 242 ± 6.6 w per m^2 ($n=27$) is not significantly different from Alexander's value ($P < 0.1$). However, caution should be exercised in interpreting this comparison of metabolic rates since a different cooling medium was used and some of Alexander's older lambs may have sucked.

Summit metabolic rate was not directly proportional to body weight but was closely related to weight raised to the power of 0.59, ie, to body surface area (Fig 5). This finding is a direct contrast to that of Alexander (1962) in lambs and to that of Mount and Stephens (1970) in newborn pigs. These workers found summit metabolism to be closely related to body weight raised to the power of 1. The comparison between merino lambs and our lambs is again confounded by the use of a different technique and lambs of different ages. However, if there is a true breed difference it would mean that the high rate of neonatal mortality in small Scottish Blackface lambs cannot be attributed to a low rate of heat production per unit body surface area as in the merino. The rate of heat production per unit body weight and therefore the requirement for food per unit body weight will be greater in small than in

large Blackface lambs and thus any interruption in milk supply will have a more rapid effect in the small lamb.

Summit metabolic rate and blood and plasma composition

Changes in blood metabolite levels caused by cold stress have already been described (Alexander 1962; Alexander and Mills 1968; Alexander *et al* 1968; Alexander *et al* 1972), but correlations between the increment in metabolic rate from basal to summit values and the associated changes in metabolite levels have not. Metabolic acidosis combined with high blood lactate or excess lactate levels is an indication of oxygen lack (Huckabee 1958; Cain 1977). Thus, the positive correlations between the increment in metabolic rate and the development of metabolic acidosis and hyperlactaemia suggest that oxygen lack did not limit heat production in lambs with low rates of summit metabolism whereas it might have done so in lambs with high rates.

Values for blood and plasma metabolite levels at birth have been reported previously (Alexander and Mills 1968; Comline and Silver 1972; Mellor and Pearson 1977) but there are no reports of any relationship between these values and summit metabolic rate. Table 2 shows that whereas body surface area alone accounted for only 13 per cent of the observed variation in the rate of summit metabolism the separate inclusion of the blood level at birth of base excess, hydrogen ions or lactate increased the accountability to 40 to 50 per cent. No similar increases in accountability were found when the birth value for either glycerol or free fatty acids was used but the use of the birth plasma glucose value did increase accountability to 23 per cent. This last finding, however, is not surprising since the birth levels of glucose and lactate were highly correlated. Both metabolic acidosis and hyperlactaemia are conditions known to result from hypoxia which is commonly encountered in the preparturient period (James *et al* 1958; Comline and Silver 1972). It thus appears that severe prepartum hypoxia is associated with a depressed heat production capacity for the first 4 h of life.

The increase in heat production observed when the newborn lamb is exposed to cold is associated with an increase in sympathetic nervous activity. The increases in the plasma levels of glucose, free fatty acids and lactate caused by cold stress are mimicked by the infusion of catecholamines into lambs under thermoneutral conditions and may be blocked in lambs exposed to cold stress by the use of adrenergic blocking drugs (Alexander *et al* 1968). When fetal lambs in late gestation were exposed to hypoxia the magnitude of the rises in the plasma

levels of glucose and free fatty acids were directly proportional to the increment in the plasma catecholamine concentration (Jones 1977). Furthermore, noradrenalin infusion into adult rats caused an increase in oxygen consumption and this increase was directly proportional to the increase in the plasma noradrenalin concentration (Depocas *et al* 1978). The increment in metabolic rate from basal to summit metabolism was positively correlated with the associated increases in the plasma concentrations of glucose, glycerol and free fatty acids and the blood concentration of lactate. This suggests that not only were plasma catecholamine concentrations increased during summit metabolism but that the increase in metabolic rate was related to the increase in plasma catecholamine concentration and thus with the level of sympathetic nervous activity. Accordingly we suggest that severe prepartum hypoxia was associated with a postpartum depression of sympathetic nervous activity and of the thermogenic response to cold stress. The present data do not enable us to postulate that the depressed thermogenic response was a direct consequence of depressed sympathetic nervous activity. Furthermore, we do not know which part of the sympathetic nervous system was depressed or whether any other part of the nervous system was similarly affected.

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Effects of colostrum on summit metabolic rate in Scottish Blackface lambs at five hours old

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The feeding of colostrum to newborn lambs led to a 17 to 20 per cent increase in summit metabolic rate estimated at five hours of age. This increase would significantly enhance the survival potential of the newborn lamb in the field. Respiratory quotient during summit metabolism and changes in plasma composition suggested that the carbohydrate content of the colostrum was a major contributor to the increase in summit metabolic rate.

THE maintenance of heat production in the newborn lamb is dependent on an adequate supply of food (Alexander 1962b) but it is not known whether feeding simply maintains heat production capacity at the level found in unfed lambs with plentiful energy reserves or whether it has an enhancing effect. Any such enhancement would increase the resistance of the newborn lamb to hypothermia. This paper describes the effects on summit metabolic rate (the maximum rate of heat production) of feeding colostrum to newborn lambs at five hours old. Blood samples were taken from some of the lambs to investigate changes in energy substrate concentrations.

Materials and methods

Animals

Pregnant Scottish Blackface ewes (mated with a Blackface ram) were brought indoors four weeks before the anticipated lambing date and were maintained in a covered yard for three weeks before being moved to individual pens. The ewes were fed *ad libitum* a proprietary complete diet (Ruminant A, Seafeld Mills). The lambs were born at an environmental temperature between 13° and 17°C, and any exhibiting obvious signs of respiratory or other distress were excluded from these experiments.

Lamb feeding

In the first experiment (natural suckling) 11 lambs remained with their dams until four hours old and 11 lambs were removed when 30 minutes old before suckling began. In the second experiment (bottle feeding) all lambs were removed from their dams when 30

minutes old. Fifteen lambs received no food during the experiment whereas 16 lambs received colostrum by bottle at a rate of 830 ml/m² body surface area (approximately 55 ml/kg). Surface areas were derived according to Peirce (1934). One half of the colostrum was given at 30 minutes old and the second half at three hours. The colostrum was obtained during the previous season from nursing ewes one to 28 hours after parturition (mean 20 hours) and was stored in plastic bottles at -20°C.

Metabolic rate

Basal metabolic rate and then summit metabolic rate were measured in all lambs when four and a half and five hours old respectively. The techniques used have already been described (Eales and Small 1980). Metabolic rate was measured by indirect calorimetry using a face mask. Basal metabolism was induced in the lamb by placing it in water at 38.5°C (thermoneutral). The steady metabolic rate which resulted after about 20 minutes of immersion in water (basal metabolic rate) was measured over a 10 minute period. Summit metabolism was then induced by progressively lowering the water temperature until the rectal temperature started to fall. The average metabolic rate during the decline of rectal temperature from 39°C to 37°C was taken as summit metabolic rate. After the measurement of summit metabolic rate the lamb was removed from the water, dried in a stream of warm air and then returned to the ewe. The subsequent development of these lambs was similar to that observed in lambs not subjected to experimentation.

Blood sampling and analysis

No blood samples were taken in the natural suckling experiment. The venae cavae of 24 lambs in the bottle feeding experiment (13 lambs in the fed group and 11 in the unfed group) were catheterised at birth via the cut umbilical veins. Duplicate 4 ml samples were taken into heparinised syringes five minutes after birth, at the end of basal metabolism and at the end of summit metabolism (rectal temperature of 37°C).

Haematocrit and red blood cell count were

estimated by conventional methods. Plasma was stored at -20°C until analysed for glucose, free fatty acids, urea and β -hydroxybutyrate according to the methods of Trinder (1969), Lauwery (1969), Marsh et al (1965) and Chandrasekaran et al (1972) respectively.

Colostrum analysis

The colostrum triglyceride concentration was estimated as triglyceride glycerol according to the method of Eggstein and Kreutz (1966) and the lactose concentration according to the method of Asatoor and King (1954). The total solid content of the colostrum was measured by freeze drying and an estimation of protein content made by subtracting the weights of triglyceride and lactose from the dry weight.

Statistical analysis

Summit metabolic rate per unit body-weight is negatively correlated with body-weight but summit metabolic rate per unit body surface area is independent of body-weight (Eales and Small 1980). Metabolic rate was therefore expressed in watts per square metre body surface area in order to exclude effects of body-weight differences. The results were treated statistically by analysis of variance and conventional Student's *t* tests and are presented as means \pm SEM.

Results

Metabolic rate and respiratory quotient

Feeding resulted in higher basal and summit metabolic rates in both the fed groups (Table 1). In the natural suckling experiment basal metabolic rate was elevated by 46 per cent ($P<0.001$) and summit metabolic rate by 20 per cent ($P<0.05$); the corresponding increases in the bottle feeding experiment were 34 per cent ($P<0.01$) and 17 per cent

($P<0.01$), respectively.

The respiratory quotient was higher during summit metabolism than during basal metabolism in all the lambs ($P<0.001$ for each of the four groups) but there were no differences between the fed and unfed groups at any time.

Blood and plasma composition in the bottle feeding experiment

The plasma levels of glucose and free fatty acids increased two-fold between birth and four and a half hours of age in the fed group ($P<0.001$ and $P<0.01$ respectively) but did not change in the unfed group (Table 2). Induction of summit metabolism was associated with increases in the concentrations of both metabolites in the fed and unfed lambs, but whereas the plasma glucose level in the fed group was significantly higher than the level in the unfed group during summit metabolism ($P<0.001$) the plasma free fatty acid level was not ($P<0.1$).

Neither feeding nor the induction of summit metabolism were associated with any significant changes in the plasma concentrations of β hydroxybutyrate or urea (mean levels were 0.09 ± 0.010 and 5.6 ± 0.26 mM respectively, $n = 24$).

There were no significant changes in haematocrit or red blood cell count during the procedures in either group and the group means were not significantly different at any time. The mean values were 41 ± 1.0 per cent and $9.02 \pm 0.151 \times 10^6/\text{mm}^3$ ($n = 24$) respectively.

Colostrum composition

The concentrations of triglyceride, lactose and protein in the colostrum were 118, 42 and 80 g per litre respectively.

Discussion

These results clearly demonstrate that feeding would be of immediate survival benefit to the newborn

TABLE 1: Effects of colostrum feeding on metabolic rate and respiratory quotient during basal and summit metabolism in five-hour-old lambs; The data is presented as mean \pm SEM

	Body-weight (kg)	Basal metabolism		Summit metabolism	
		Metabolic rate (W/m ²)	Respiratory quotient	Metabolic rate (W/m ²)	Respiratory quotient
Maternal feeding					
Fed lambs (n = 11)	4.25 ± 0.239	64 ± 3.2	0.83 ± 0.011	296 ± 11.3	0.90 ± 0.008
Unfed lambs (n = 11)	4.08 ± 0.253	44 ± 2.2	0.87 ± 0.022	247 ± 13.5	0.92 ± 0.019
Group difference	NS	$P<0.001$	NS	$P<0.05$	NS
Bottle feeding					
Fed lambs (n = 16)	4.26 ± 0.205	63 ± 2.4	0.88 ± 0.014	283 ± 8.5	0.95 ± 0.009
Unfed lambs (n = 15)	3.85 ± 0.190	47 ± 3.9	0.85 ± 0.024	243 ± 9.5	0.96 ± 0.015
Group difference	NS	$P<0.01$	NS	$P<0.01$	NS

NS Not significant

TABLE 2: Plasma composition (mean \pm SEM) at birth and during basal and summit metabolism in fed ($n = 13$) and unfed ($n = 11$) lambs

		Birth	Plasma composition Basal metabolism	Summit metabolism
Glucose (mM)	Fed lambs	3.19 \pm 0.364	6.42 \pm 0.490	10.7 \pm 0.55
	Unfed lambs	2.61 \pm 0.317	2.88 \pm 0.577	7.70 \pm 0.549
	Group difference	NS	P < 0.001	P < 0.001
Free fatty acids (m equiv per litre)	Fed lambs	0.46 \pm 0.091	0.90 \pm 0.126	1.52 \pm 0.117
	Unfed lambs	0.53 \pm 0.133	0.50 \pm 0.048	1.18 \pm 0.116
	Group difference	NS	P < 0.05	NS

NS Not significant

lamb. The increase of nearly 20 per cent in summit metabolic rate (Table 1) would result in a reduction of the lower temperature survival limit of the lamb by approximately 10°C (Alexander 1962a).

The depletion of body energy reserves in unfed lambs during the first five hours of life does not lead to a depression of summit metabolic rate (Eales and Small 1980). Thus the higher summit metabolic rate observed in the fed lambs was related to a specific thermogenic effect of colostrum and not to a repletion of body energy reserves. The absence of a change in haematocrit or red blood cell count in the fed lambs suggests that the fluid content of the colostrum did not contribute to an expansion of plasma volume and consequently to an increase in circulatory efficiency. It thus seems probable that the energy substrate content of the colostrum was responsible for the increase in summit metabolic rate. When colostrum is fed to the newborn lamb the plasma concentrations of immunoglobulin and glucose increase within one hour indicating that the ingested colostrum is absorbed quickly from the gut (Comline and Silver 1972, Mellor and Pearson 1977). Summit metabolic rate was estimated in the bottle fed lambs four and a half hours after the first feed and two hours after the second and the plasma glucose concentration in the fed lambs during basal metabolism was twice that in the unfed lambs ($P < 0.001$). It is thus reasonable to assume that a significant amount of the ingested energy substrate had been absorbed.

In the bottle feeding experiment summit metabolic rate in the fed lambs was 40 W/m² higher than in the unfed lambs (Table 1). If we assume that this elevation of metabolic rate was present throughout the whole five hour period from birth to the end of summit metabolism, a gross overestimation, we can calculate that the extra energy expended would have been 0.59 MJ/m². Analysis of the colostrum showed that the total energy given to the lambs was 5.55 MJ/m² body surface area, 0.60 MJ/m² being derived from carbohydrate, 3.81 MJ/m² from lipid and 1.14 MJ/m² from protein (assuming the calorific values of these substrates to be 17.2, 38.9 and 17.2 kJ/g respectively; White et al 1964). It is thus clear that each

of the three major substrates in the colostrum was present in an adequate amount to account solely for the increased rate of heat production.

In all lambs during the period from basal to summit metabolism there was a significant increase in respiratory quotient but there were no significant changes in the plasma levels of urea and β -hydroxybutyrate. These observations and the high respiratory quotient during summit metabolism suggest that carbohydrate catabolism was the major contributor to heat production and that fat and protein catabolism were of limited significance.

The elevation of summit metabolic rate observed in the fed lambs was associated with a plasma glucose level markedly higher than that observed in the unfed lambs. Thus the colostrum induced elevation of summit metabolic rate could have been a consequence of an increased supply of carbohydrate substrate to the heat producing tissues.

The increase in basal metabolic rate observed in the fed lambs is an example of the 'specific dynamic effect' of food ingestion first identified by Rubner (1902). The exact biochemical mechanism of this effect is uncertain but it would appear that feeding has a similar qualitative effect on heat production in the newborn lamb as it does in adult sheep (Thompson et al 1978) and in adult and infant humans (Garrow and Hawes 1972, Alvear and Brooke 1978).

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Determinants of Heat Production in Newborn Lambs

by

F. A. Eales* and J. Small

ABSTRACT. — Measurement of summit metabolism (the maximum rate of heat production) in lambs aged 1 or 4h revealed considerable between animal variation. Summit metabolism per unit body weight decreased as body weight increased whereas summit metabolism per unit body surface area was independent of body weight. Severe pre-partum hypoxia was apparently associated with a low summit metabolism at 1 or 4h of age which made such lambs very susceptible to hypothermia. This deficiency in heat production capacity did not appear to be a permanent feature since most lambs so affected recovered full thermoregulatory ability by 12h of age. Feeding of colostrum conferred an immediate 18% increase in summit metabolism. The significance of these findings to the prevention of hypothermia in the newborn lamb is discussed.

INTRODUCTION

Hypothermia is a major cause of death in newborn lambs, accounting for up to half of all losses which can exceed 25% of the yearly lamb crop. Hypothermia occurs when heat loss exceeds heat production. Thus, any deficiency in heat production capacity will lead to an increased susceptibility to hypothermia. The work of Alexander (1962) has demonstrated considerable variation in heat production capacity between lambs of a single breed but extensive studies failed to reveal the source of this variation (Alexander, 1970; Alexander and Bell, 1975a; Alexander and Bell, 1975b; Alexander and Williams, 1970; Alexander, Bell and Hales, 1972; Alexander, Mills and Scott, 1968). The object of the present work was to establish whether similar between lamb variation in heat production capacity existed in British lambs and if it did to investigate factors which might contribute to this variation.

MEASUREMENT OF HEAT PRODUCTION

In order to compare heat production capacity in different lambs it is necessary to define the rates of heat production which are to be measured. There are two rates which are characteristic of the individual lamb at any particular time. These are basal metabolism, the lowest rate of heat production observed under thermoneutral conditions, and summit metabolism, the highest rate which is induced by cold stress adequate to cause a slow fall in

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rectal temperature. In order to measure these two rates of metabolism a technique is required which will create two conditions, thermoneutrality and cold stress. The thermoneutral state was obtained by placing the lamb in a water tank (Fig. 1) maintained at

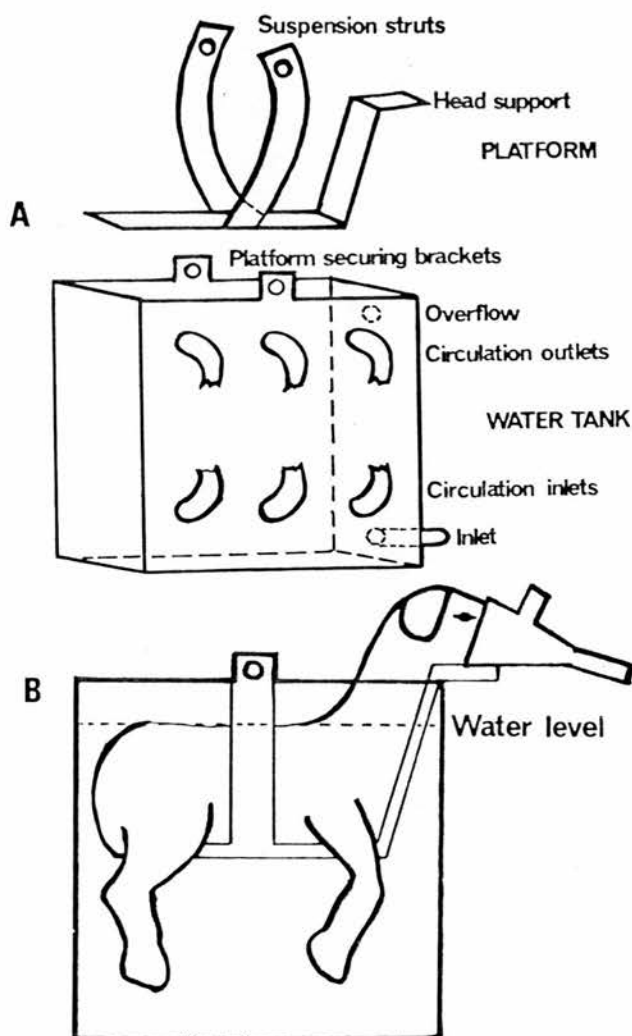


Fig. 1. The water tank and lamb supporting platform (A) and a lamb in the water tank showing the supporting platform and face mask in place (B).

38.5° C and cold stress was induced by cooling the water until rectal temperature commenced to fall. Oxygen consumption and carbon dioxide production were estimated by indirect calorimetry using a face mask and the rate of heat production was calculated from these values according to Brouwer (1965). A typical reaction to this procedure is shown in Fig. 2. After the estimation of basal metabolism the water was cooled and both metabolic rate and rectal temperature increased. Eventually metabolic rate reached a maximum (summit metabolism) and rectal temperature slowly fell.

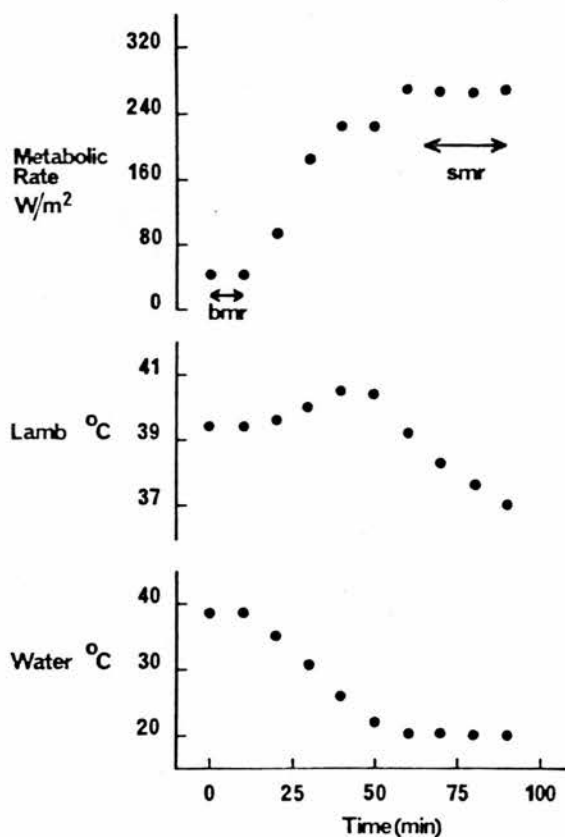


Fig. 2. Metabolic rate, rectar temperature and water tank temperature during the measurement of basal metabolic rate (bmr) and summit metabolic rate (smr) in a 4-h old Blackface singleton lamb of 4.60 kg bw.

Lambs were catheterised by an umbilical vein at birth in order to monitor changes in blood composition. Samples were taken immediately after catheterisation and throughout the experimental procedures.

VARIATION IN SUMMIT METABOLISM

Figure 3 shows data obtained from 29 Scottish Blackface and Scottish Blackface cross lambs in which summit metabolism was estimated within 4h of birth. None of the lambs had sucked. Clearly considerable between lamb variation in summit metabolism existed in these lambs. The lambs with very low summit metabolism were particularly susceptible to hypothermia. The mean rate of summit metabolism was 16.5 ± 0.7 W/kg ($n = 29$) which was significantly lower than that of 19.8 ± 0.45 W/kg ($n = 19$) found by Alexander (1962) in Merino lambs aged 9h or less ($P < 0.001$). Of great interest was the much higher variation around the mean found in our lambs. Calculation of the coefficients of variation for the two sets of data yielded values of 20% for our lambs and 10% for those of Alexander. Since summit metabolism was estimated at earlier ages in our lambs than in

those of Alexander this might suggest that the higher variation found was associated with a circumstance or event occurring at or before birth the influence of which decreased with age.

SUMMIT METABOLISM AND BODY WEIGHT

Heat loss is proportional to surface area and the surface area to body weight (SA/W) ratio increases as the body weight of the newborn lamb decreases (Peirce, 1934 quoted by Alexander, 1962). Thus, in order to compensate for its large SA/W ratio the small lamb must produce more heat per unit body weight than the large lamb. Alexander (1962) demonstrated that the summit metabolism per unit body weight was the same in small and large Merino lambs and offered this as an explanation of the higher mortality from hypothermia in small lambs. However, our work in Scottish Blackface lambs (Fig. 3)

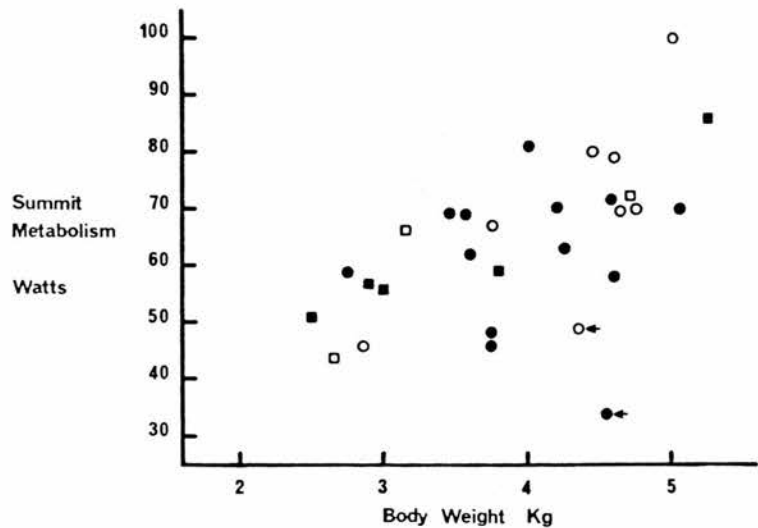


Fig. 3. Relation of summit metabolism to bodyweight in 29 lambs aged 1 or 4 h. The 2 lambs marked with arrows showed low summit metabolisms and atypical responses to cold stress and were omitted from analysis of the data. The round symbols represent Blackface lambs and the square symbols Blackface cross lambs. A closed symbol indicates measurement of metabolic rates at 1 h of age and an open symbol measurement at 4 h.

demonstrated that summit metabolism per unit body weight increased as body weight decreased (Fig. 4a), such that summit metabolism was directly proportional to surface area (Fig. 4b). Why then is the incidence of mortality from hypothermia higher in the small Scottish Blackface lamb (Purser and Young, 1964)? The rate of energy consumption per unit body weight in small lambs will be higher than in the large lamb maintained under similar conditions so that any interruption in the milk supply will have a more acute effect in the small lamb. The large lamb is often a singleton whereas the small lamb is commonly one of twins or triplets. Poor nutrition is more common in twins and triplets which must compete for their milk supply. Thus, the higher mortality in small Scottish Blackface lambs may be related more to litter size than to body weight.

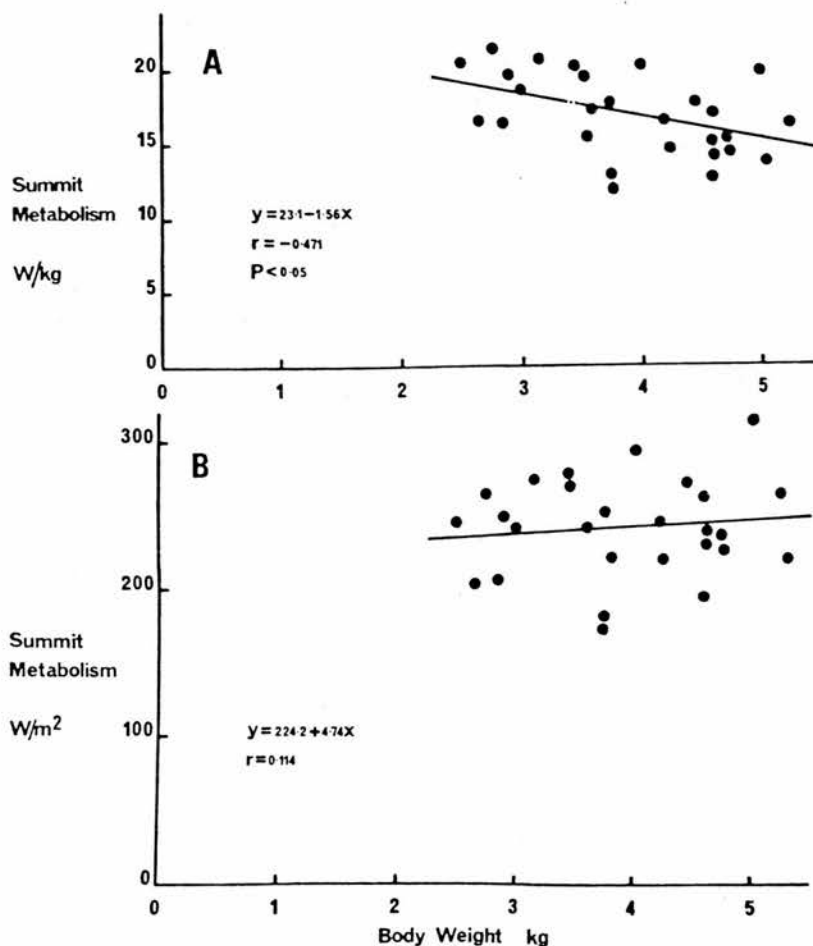


Fig. 4. Relation of summit metabolism per unit body weight to body weight (A) and per unit body surface area to body weight (B) in 27 lambs aged 1 or 4 h. Surface area (m²) was derived according to Peirce (1934): $= 0.121 \times \text{bw (kg)}^{0.59}$.

SUMMIT METABOLISM AND PRE-PARTUM HYPOXIA

Blood samples were taken from 19 of the lambs shown in Fig. 3 to examine their metabolic state at birth and to follow changes in blood composition during thermoneutrality and then during cold stress. The results obtained are shown in Table 1. The values for blood base excess, blood hydrogen ions and blood lactate at birth suggest that all lambs suffered some degree of hypoxia before birth (Cain, 1977; Huckabee, 1958; Jones, 1977). A shortage of oxygen leading to tissue hypoxia is associated with increases in blood lactate and excess lactate and a decrease in base excess (Huckabee, 1958). Thus the correlations between the increment in metabolic rate from basal to summit metabolism and the changes in acidbase status and blood lactate and excess lactate indicate that oxygen supplies did not limit heat production in lambs with low summit metabolisms whereas it might have done so in lambs with high rates. The corresponding positive correlations with the increases in the plasma

Table 1. Blood composition in 19 lambs subjected to cooling (mean \pm SE). The significances of the changes from birth to basal metabolism and from basal to summit metabolism are shown at the latter period. The right hand column shows correlations between the increment in metabolic rate from basal to summit levels and the corresponding changes in blood and plasma metabolite levels.

	Birth	Basal metabolism	Summit metabolism	Correlations between the increment in metabolic rate from basal to summit levels and the corresponding changes in blood and plasma metabolite values
Blood base excess (meq/l)	-2.3 \pm 1.75	3.4 \pm 1.53 ***	-3.5 \pm 0.99 ***	-0.861 ***
Blood hydrogen ions (g ion/l) 10^{-8}	6.26 \pm 0.589	4.58 \pm 0.225 ***	6.10 \pm 0.164 ***	0.719 ***
Blood lactate (mmol/l)	5.82 \pm 0.943	3.91 \pm 0.655 ***	5.78 \pm 0.456 ***	0.626 ***
Blood excess lactate (mmol/l)	—	0	1.8 \pm 0.38 ***	0.729 ***
Plasma glucose (mmol/l)	3.3 \pm 0.36	2.6 \pm 0.34 *	5.4 \pm 0.30 ***	0.467 *
Plasma glycerol (μ mol/l)	117 \pm 21.9	150 \pm 35.4	509 \pm 50.5 ***	0.606 **
Plasma free fatty acids (mmol/l)	0.41 \pm 0.076	0.38 \pm 0.029	1.36 \pm 0.082 ***	0.454 *

levels of glucose, free-fatty acids and glycerol show that mobilisation of these metabolites was high in lambs showing a large increase in metabolic rate on cooling and low in lambs showing a small increase. This suggests a relationship between summit metabolism and the level of sympathetic nervous activity (Alexander, Mills and Scott, 1968).

The relationship between the metabolic state at birth and summit metabolism was examined by multiple regression analysis as shown in Table 2 ($n = 19$). Whereas surface area

Table 2. The effect of body surface area and blood and plasma composition at birth on summit metabolism measured at either 1 or 4 hours of age in 19 lambs. The regression equations are of the form $y = a + b x_1 + c x_2$ where y represents summit metabolism (W), x_1 body surface area (m^2) and x_2 the concentration of the metabolite.

Metabolite	a Constant	b Body surface area	c Metabolite concentration	Percentage of variation accounted for by coefficients
None	— 3.05	239.7		13
Blood lactate (mM)	—27.33	377.6	—2.41	49
Base excess (meq/l)	—18.15	302.9	1.09	40
Blood hydrogen ions (g ion/l) 10^{-8}	2.12	304.7	—3.71	50
Plasma glucose (mM)	—27.52	379.7	—4.31	23
Plasma glycerol (mM)	1.10	216.3	19.91	9
Plasma free fatty acids (meq/l)	—14.11	260.7	12.76	16

alone only accounted for 13% of the variation in summit metabolism, inclusion of the blood level at birth of base excess, hydrogen ions or lactate increased the accountability to approximately 50%. As indicated above metabolic acidosis and hyperlactaemia are consequences of hypoxia and thus it is probable that severe pre-partum hypoxia was associated with a low summit metabolism and an increased susceptibility of hypothermia. However, if hypothermia was prevented and the lambs were adequately fed recovery of full body function was usually achieved within 12h of birth. These effects of hypoxia therefore appear to be temporary in nature.

These laboratory observations are supported by field data. Acid-base status was measured in 75 lambs born in a covered yard at the Moredun Institute. All three lambs which died in the first 6 h of life exhibited a severe metabolic acidosis at birth (Fig. 5).

FEEDING AND SUMMIT METABOLISM

The effects of feeding on summit metabolism were examined by comparing summit metabolism values in two groups of lambs, one fed colostrum between 1 and 3 h of age and the other not. At 4 h of age basal metabolism and then summit metabolism were estimated. Basal metabolism was 37% higher in the fed group and summit metabolism was 18% higher (Table 3). In both groups the respiratory quotient (RQ) was higher during summit metabolism than during basal metabolism and indicated that most of the heat produced during summit metabolism was derived from carbohydrate catabolism. The effects of

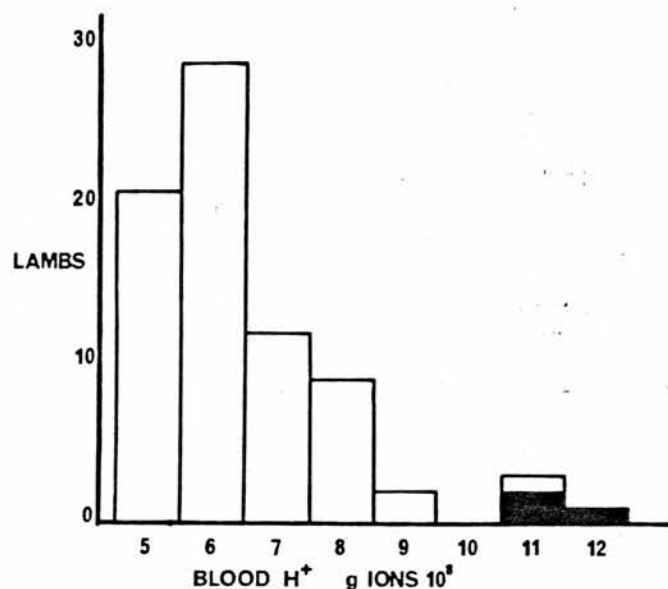


Fig. 5. The distribution of blood hydrogen ion levels at birth in 75 lambs. The three lambs which died within 6 h of birth are denoted by dark shading.

Table 3. The effects of feeding on basal metabolism and summit metabolism in newborn lambs (mean \pm SE).

		Basal metabolism		Summit metabolism	
		Metabolic rate (W/m ²)	RQ	Metabolic rate (W/m ²)	RQ
Fed	(n = 27)	63 \pm 1.9	0.86 \pm 0.011	288 \pm 6.8	0.93 \pm 0.008
Starved	(n = 26)	46 \pm 2.4	0.86 \pm 0.016	245 \pm 7.8	0.94 \pm 0.012

Table 4. The effects of feeding on plasma composition during basal metabolism and summit metabolism (mean \pm SE)

		Plasma composition during:	
		Basal metabolism	Summit metabolism
Fed lambs (n = 13)	Plasma glucose (mM)	6.42 \pm 0.490	10.69 \pm 0.548
	Plasma free fatty acids (meq/l)	0.90 \pm 0.126	1.52 \pm 0.117
	Plasma glucose (mM)	2.88 \pm 0.577	7.70 \pm 0.549
Starved lambs (n = 11)	Plasma free fatty acids (meq/l)	0.50 \pm 0.048	1.18 \pm 0.116

feeding on blood composition are shown in Table 4. During basal metabolism the mean levels of both glucose and free-fatty acids in the fed group were significantly higher than those in the starved group ($P < 0.001$ and $P < 0.05$ respectively). Cold stress caused levels of both metabolites to rise in each group, but whereas the group difference in plasma free-fatty acids disappeared ($P < 0.1$) that in plasma glucose was maintained during summit metabolism ($P < 0.001$). These observations suggest that during summit metabolism when carbohydrate was apparently the major source of energy the availability of glucose may have limited heat production in the lambs. Carbohydrate reserves in the newborn lamb are small, estimated at about 1% of bodyweight (Shelley, 1960). If high plasma levels of glucose are necessary to maintain a high rate of heat production it is evident that the early initiation of feeding by the newborn lamb is an essential prerequisite to the maintenance of thermoregulatory ability.

CONCLUSION

Factors which increase or decrease heat production are shown in Fig. 6, together with those affecting heat loss and the consequences of hypothermia itself. Poor foetal nutrition is included because it is likely to depress heat production since lambs born to ewes subjected to undernutrition during pregnancy have been found to possess very low body energy reserves (Alexander, 1962).

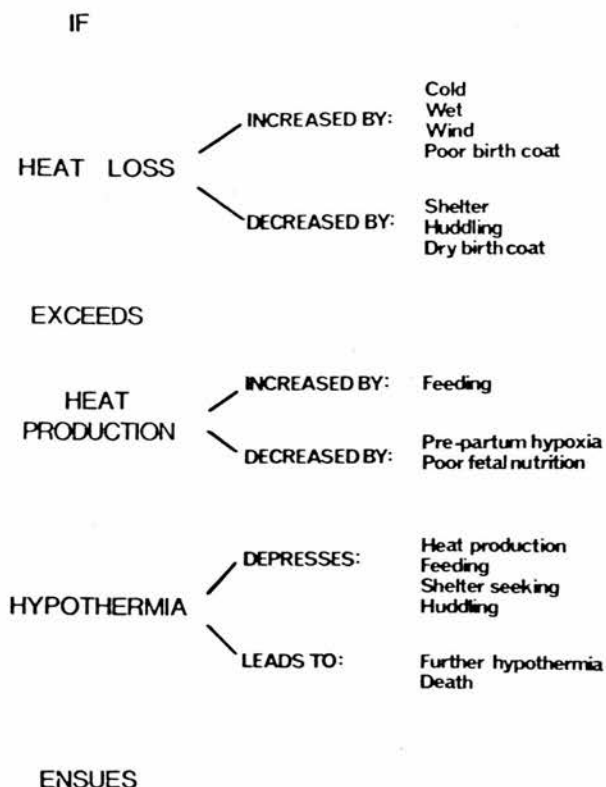


Fig. 6. Factors which increase or decrease the susceptibility of the newborn lamb to hypothermia.

Hypothermia in the newborn lamb cannot be attributed to any single factor. The incidence of this condition can only be reduced if the multi-factorial nature of its aetiology is fully understood.

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Plasma composition in hypothermic lambs

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POST mortem examinations of new born lambs which are thought to have died from hypothermia commonly reveal an exhaustion of body energy reserves and an empty alimentary tract. Such deaths are normally attributed to the "starvation/exposure" syndrome (Johnston 1977). However, this diagnosis does not specify whether the condition occurred because of excessive heat loss or deficient heat production. Plasma composition in 20 live hypothermic lambs aged from 12 hours to one week was examined in an attempt to investigate which of these two factors was the more important cause of the hypothermia.

Twenty hypothermic lambs (one Scottish blackface singleton, seven Scottish blackface twins, 10 Cheviot twins and two Cheviot triplets) were identified by the shepherd in the field and were presented for examination. Mean rectal temperature was $30 \pm 1.0^\circ\text{C}$ (range 16.0 to 36.6°C). A blood sample was withdrawn immediately from a jugular vein into a heparinised syringe. The plasma was separated by centrifugation and stored at -20°C . The concentrations of glucose, free fatty acids and lactate were estimated using the methods described by Trinder (1969), Lauwery (1969) and Hohorst (1963) respectively. Plasma gamma globulin concentration was estimated by the method of Mancini and others (1965) as modified by Fahey and McKelvey (1965).

The mean body-weight of the lambs was 2.8 ± 0.20 kg, considerably less than the mean weight of 3.4 kg which was calculated from a representative sample of whole flock data, the sample containing the same proportions of singletons, twins and triplets of each breed. The rate of energy consumption per unit body-weight in the small lamb is greater

than in the large lamb maintained under similar conditions (Eales and Small 1980). Thus the rate of depletion of body energy reserves in the lambs which became hypothermic would have been proportionally greater than in the heavier normothermic lambs.

The low value for the plasma concentration of gamma globulins (Table 1) strongly suggests that the lambs were starving and the low values for the plasma concentrations of glucose and free fatty acids suggest that body energy reserves, especially those of carbohydrate, were exhausted. It would appear that starvation and exhaustion of body energy reserves led to a depression of heat production which led finally to hypothermia (Alexander 1962). The low plasma lactate concentration (Table 1) which indicates a low metabolic rate in the period previous to sampling (Alexander and others 1972, Eales and Small 1980) supports this suggestion.

This diagnosis is more useful than simply the "starvation/exposure" syndrome. The provision of additional shelter for these lambs may have delayed the onset of hypothermia but it would not have prevented it. However it is likely that the condition would have been prevented in most of the lambs by ensuring that they had received an adequate and continuous supply of colostrum or other suitable food.

Acknowledgements.—We are grateful to all the staff at Sourhope who so freely gave of their time and energy during the conduct of this work. We thank C. Burrells for the gamma globulin estimations.

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TABLE 1: Plasma composition in hypothermic lambs. Values from healthy normothermic Scottish blackface lambs are included for comparative purposes. The data is presented as mean \pm SEM

	n	Age (h)	Environment during sampling	Plasma composition			
				Glucose (mM)	Free fatty acids (mM)	Lactate (mM)	Gamma globulins (g/litre)
Hypothermic lambs	20	45 \pm 9.7	Ambient	0.6 \pm 0.11	0.4 \pm 0.07	2.3 \pm 0.33	1.3 \pm 0.7
Normothermic lambs	12†	24	Ambient	7.2 \pm 0.41			
	19*	2 \pm 0.3	Thermoneutral	2.6 \pm 0.34	0.4 \pm 0.03	3.9 \pm 0.66	38 \pm 5.0
	19*	2 \pm 0.3	Acute cold	5.4 \pm 0.30	1.4 \pm 0.08	5.8 \pm 0.46	

* Eales and Small (1980)

† Mellor and Pearson (1979)

Papers and Articles

Causes of hypothermia in 89 lambs

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Veterinary Record (1982) **110**, 118-120

The causes of hypothermia in 89 lambs were identified on the basis of history and clinical biochemistry. Excessive heat loss accounted for 24 per cent of the cases, and depressed heat production because of either severe hypoxia during birth, immaturity or starvation accounted for 72 per cent. Exhaustion of energy reserves and hypoglycaemia were marked characteristics of lambs which became hypothermic after 12 hours of age. Most of the lambs were either twins or triplets. The implications of the findings for both the treatment and prevention of hypothermia in newborn lambs are discussed.

THERE are two well described causes of hypothermia in newborn lambs: excessive heat loss due to inclement climatic conditions (Alexander 1964, Eales and Small 1980) and depressed heat production due to starvation (Alexander 1962, Eales and others 1980). In addition, there are at least two factors which increase the susceptibility of a newborn lamb to hypothermia. These are severe hypoxia during birth which results in depressed heat production after birth (Eales and Small 1980) and immaturity, which is commonly associated with depressed heat production capacity (Dawes and Parry 1965, Forfar and Arneil 1978).

Post mortem examination of lambs which have died from hypothermia does not enable a specific diagnosis of cause to be made in most cases. The tentative diagnosis of 'starvation exposure' is thus commonly reported. In order to propose regimes for both prevention and treatment of this condition a more definite elucidation of the causes is required. This is only possible if clinical biochemistry is employed. An attempt to diagnose the causes of hypothermia in 89 affected lambs on the basis of clinical biochemistry and history is reported here.

Materials and methods

Eighty-nine hypothermic lambs (36 Suffolk cross, 33 Cheviot, 14 Scottish blackface, four Texel cross, one Border Leicester cross and one Dorset cross) aged between 30 minutes and seven days were presented for examination. A 2ml blood sample was withdrawn immediately from a jugular vein into a heparinised syringe. The haematocrit value was determined by the capillary tube centrifugation method. The plasma was separated by centrifugation and stored at -20°C . The concentrations of glucose, lactate and copper were estimated using methods described by Trinder (1969), Hohorst (1963) and Suttle (1981) respectively. Plasma gammaglobulin concentration was estimated by the method of Mancini and others (1965) as modified by Fahey and McKelvey (1965). The results are presented as mean \pm se.

Resuscitation was attempted using warm air and in some cases an intraperitoneal injection of glucose solution (Eales and others 1982). Post mortem examinations were performed on 15 of the 32 lambs which died during or soon after resuscitation. Brain, spinal cord and portions of liver and lung

were removed from all carcasses and placed in Baker's fixative together with any other tissues showing gross abnormality. Selected blocks of these were processed for light microscopy. Paraffin sections (12 μm) from brain and spinal cord were stained by the Luxol fast blue method and frozen sections (20 μm) by the osmium tetroxide-alpha naphthylamine method. Paraffin sections of all tissues (6 μm) were stained by haematoxylin and eosin and of liver alone by the periodic acid-Schiff method.

Diagnosis

The major criteria used in diagnosis are shown in Table 1. Hypothermia due to excessive heat loss was diagnosed in lambs with a history of exposure, a normal plasma concentration of gammaglobulin (related to age), but elevated levels of glucose and lactate, which are associated with a high rate of heat production in response to a high rate of heat loss (Eales and Small 1980).

Severe hypoxia during birth was implicated as a major factor contributing to hypothermia in lambs aged 12 hours or less which had a basal plasma concentration of glucose, indicating a low rate of heat production before the hypothermia, but a high concentration of lactate, a product of the hypoxic period (Eales and Small 1980).

Immaturity and consequently low heat production capacity was implicated as a major factor contributing to hypothermia in lambs which either: had high haematocrit values, suggestive of chronic placental hypoxia and likely placental insufficiency (Mellor and Pearson 1977, Robinson and others 1979); or were known to be premature; or were aged four hours or less and showed basal levels of both glucose and lactate, suggesting that the lamb had not responded to a high rate of heat loss by an increase in heat production (Eales and Small 1980).

Hypothermia caused by depressed heat production because of starvation was diagnosed in lambs aged six hours or more which had low plasma concentrations of glucose, lactate and gammaglobulins (Alexander 1962, Eales and others 1980).

TABLE 1: Criteria employed in the diagnosis of cause of hypothermia

Diagnosis	History	Biochemistry
Excessive heat loss	Exposure	Plasma glucose >3.0 mM Plasma lactate >6.0 mM Plasma gammaglobulins appropriate for age (Fig 2)
Depressed heat production caused by:		
severe hypoxia during birth	Age <12 hours	Plasma glucose <4.0 mM Plasma lactate >10.0 mM
immaturity	Gestational age <140 days	Any age: haematocrit >50 per cent In lambs aged <4 hours plasma glucose <3.0 mM and plasma lactate <6.0 mM
starvation	Age >6 hours	Plasma glucose <2.0 mM Plasma lactate <6.0 mM Plasma gammaglobulins low for age (Fig 2)

TABLE 2: Distribution of hypothermic lambs according to age and cause of hypothermia

Diagnosis	Lamb age (hours)							All ages
	0 to 2	2 to 4	4 to 8	8 to 16	16 to 24	24 to 48	over 48	
Excessive heat loss	6	10	3	1	1	0	0	21
Depressed heat production caused by:								
severe hypoxia during birth	6	2	0	1	0	0	0	9
immaturity	1	8	4	4	6	1	0	24
starvation	0	0	0	3	11	9	8	31
All causes	13	20	7	9	18	10	8	85

Results

The numbers of lambs in each diagnostic category are shown in Table 2 and the incidence of hypothermia related to age is shown in Fig 1. Excessive heat loss accounted for 21 cases (24 per cent) and was mainly a characteristic of the first four hours of life. Starvation accounted for 31 cases (35 per cent) and was mainly found in lambs aged 16 hours or more. Severe hypoxia during birth was a major factor contributing to hypothermia in nine cases (10 per cent) mostly occurring in the first two hours of life. Immaturity was associated with 24 cases (27 per cent) and was found in lambs of all ages. Six of these lambs weighed less than 2 kg and five weighed between 2 and 2.5 kg. Four cases were not attributed to the causes shown in Table 1. Hypothermia was caused in these lambs by multiple rib fractures in two cases, intestinal obstruction in one case and by acute phenol poisoning in the other (Eales and others 1981).

The mean plasma concentrations of glucose, lactate and gammaglobulins related to age at presentation are shown in Fig 2. High values for the plasma concentrations of glucose and lactate were observed in lambs which became hypothermic in the first few hours of life, but as age of presentation increased these values decreased. Very low plasma gammaglobulin values were observed in all the lambs. The mean haematocrit value was 46 ± 0.4 per cent ($n = 75$). The mean plasma concentration of copper in lambs aged 24 hours or less on presentation was 0.18 ± 0.025 mg/litre ($n = 24$).

The mean values for lamb bodyweight, litter size and rectal temperature on presentation were 3.2 ± 0.09 kg, 2.2 ± 0.06 lambs and $29.6 \pm 0.52^\circ\text{C}$ ($n = 89$) respectively.

The results of post mortem examination of 15 lambs which were not successfully resuscitated are shown in Table 3. Neuropathological changes were restricted to lambs previously diagnosed as immature according to the criteria in Table 1.

Discussion

Fig 1 demonstrates that there were two ages at which hypothermia was likely to occur (birth to five hours, and 12 to

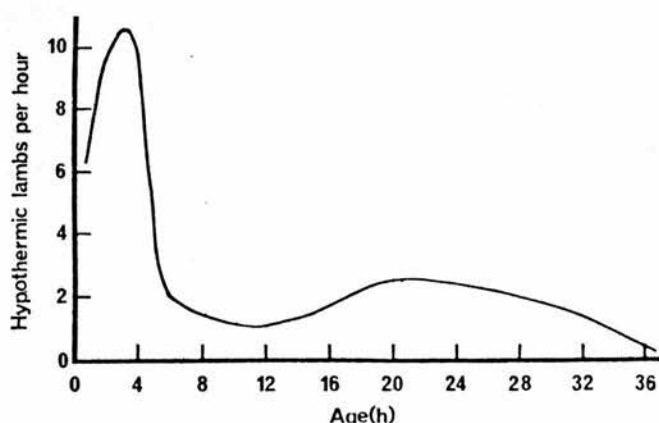


FIG 1: Distribution of hypothermic lambs according to age

36 hours) and Table 2 shows that a diagnosis of starvation-exposure would be inappropriate in most cases. The major cause of hypothermia in the first period was excessive heat loss presumably related to exposure of the wet newborn lamb to inclement climatic conditions. In addition some lambs became hypothermic because of depressed heat production related either to severe hypoxia during birth or to immaturity. Depletion of energy reserves was not a characteristic of lambs becoming hypothermic in this period. Hypothermia in the second period (12 to 36 hours) was associated with starvation, depleted energy reserves and a low rate of heat production exacerbated in some cases by immaturity. In view of the serious hypoglycaemia found in these lambs it would seem prudent, before rewarming, to administer glucose by a route from which it would be quickly absorbed (Eales and others 1982).

Immaturity was diagnosed as an important factor contributing to hypothermia in 24 cases and the post mortem findings support this diagnosis. Three specific neurological abnormalities were found only in the lambs which had been previously diagnosed as immature. Non-myelinated areas were found in the corpus callosum, cerebral gyri and the folian cores of the vermis where myelination should have commenced by 140 days of gestation (Romanes 1947, Barlow

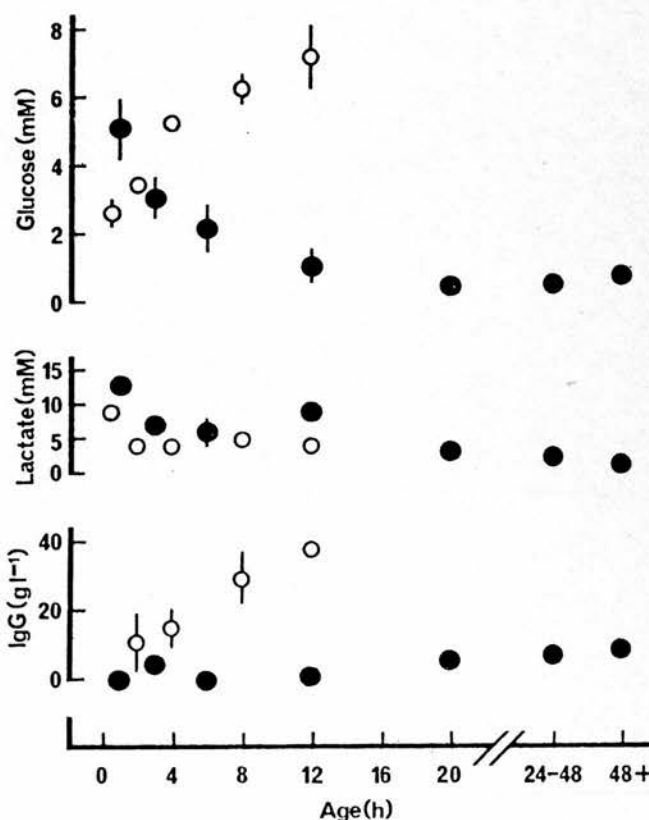


FIG 2: Plasma concentrations (mean \pm se) of glucose, lactate and gammaglobulins (IgG) in 89 hypothermic lambs related to the ages at which they become hypothermic. The open circles show mean values from 18 healthy well fed Scottish blackface lambs (Mellor and Pearson 1977)

TABLE 3: Distribution of neuropathological changes and retention of fetal lung characteristics in 15 lambs according to the diagnosis of cause of hypothermia based on history and biochemistry (Table 1)

Diagnosis	n	Non-myelination	Porencephaly	Periventricular sinus dilation	Retention of fetal characteristics in lungs
Severe hypoxia during birth	1	0	0	0	0
Immaturity	6	5	2	2	3
Starvation	6	0	0	0	2
Trauma	2	0	0	0	0

1969). These lambs were either premature or they were born after 140 days of gestation, but myelination was delayed. Porencephaly or white matter cavitation is a change which reflects some insult to the fetus during development. The significance, if any, of dilated periventricular sinuses is unknown. Retention of fetal characteristics in the lungs of some of the lambs also reflects immaturity (Reynolds and Strang 1966).

Most of the hypothermic lambs were either twins or triplets (mean litter size 2.2 ± 0.06 lambs from flocks with a mean litter size of about 1.4 lambs). This suggests that litter size may have been an important determinant of susceptibility to hypothermia. There are a number of factors which may account for this observation. The newly lambled ewe will take longer to lick twins or triplets dry than it would a single lamb and heat loss will thus be greater. Twins and triplets take longer to stand and suck than do single lambs (Bareham 1976) and thus the increase in heat production capacity that early sucking confers (Eales and Small 1981) will be delayed. Twins and triplets have a higher rate of heat production per unit bodyweight than does the larger single lamb and thus energy reserves will be exhausted faster (Eales and Small 1980). The milk requirement of a set of twins or triplets considerably exceeds that of a single lamb and thus starvation is more likely. Finally, immaturity is more likely in twins and triplets (Alexander 1974).

The mean temperature of the lambs on presentation was $29.6 \pm 0.52^\circ\text{C}$ ($n = 89$). This suggests that in many cases the shepherd, making subjective assessments based on behaviour and appearance, was unable to detect the condition in the early stages. Lambs suffering hypothermia of only a few degrees often appear merely weak and in no immediate danger, even though their temperatures may be dropping rapidly. Shepherds should be urged to take the temperature of any lamb which appears abnormal in any way in order to detect the condition in the early stages and to initiate resuscitation, which will be most effective if the drop in temperature is minimised.

The mean plasma concentration of copper in the hypothermic lambs aged 24 hours or less of 0.18 ± 0.025 mg/litre ($n = 24$) was significantly less than the mean plasma value of 0.55 ± 0.028 mg/litre ($n = 3$) ($P < 0.01$) and the mean blood value of 0.58 ± 0.012 mg/litre ($n = 8$) ($P < 0.001$) found in Clun Forest lambs 24 hours old by Howell and others (1968). It is also less than the mean value of 0.34 ± 0.020 mg/litre ($n = 10$) ($P < 0.001$) found in newborn unsuckled Scottish blackface lambs by Eales and Small (unpublished data). This low plasma copper level suggests that hypocuprosis may be associated with increased susceptibility to hypothermia and this finding merits further investigation.

Three findings emerge from our observations which have application to the prevention of hypothermia. First, if most cases occur within a few hours of birth excessive heat loss is likely to be the major cause and more shelter is required. Second, if most cases occur in lambs aged 12 hours or more starvation is likely to be the major cause and attention should be directed to the nutrition of the lambs and the ewes. Finally, it is inevitable that some lambs will be born with poor thermoregulatory ability and these lambs must be provided with adequate shelter and nutrition.

Acknowledgements.—We are grateful to all the staff at Cardrona Mains, Peebles and at Sourhope for their cheerful cooperation during lambing. We thank our many colleagues at the Moredun Research Institute and at the Hill Farming Research Organisation who have contributed to this study.

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Cell adherence of *Dictyocaulus viviparus* larvae

BECAUSE there appears to be no correlation between circulating eosinophils and immunity to helminths, the in vitro activity of whole blood and serum containing eosinophils was investigated, using larvae of *Dictyocaulus viviparus*. Results of four experiments indicated that though larvae incubated away from blood were mobile, those incubated in the presence of blood were immobile and surrounded by densely packed mononuclear and polymorphonuclear cells. Cells adherent to larvae were eosinophils. Larvae incubated in the absence of blood cells were mobile after 48 hours but incubation with blood cells immobilised the larvae within two hours and larvae were surrounded by eosinophils at three hours. No mesophils survived 48 hours' incubation but 45 per cent of eosinophils, 27 per cent of lymphocytes and 22 per cent of neutrophils did survive. Eosinophils adhered to larvae in all cultures containing unheated serum but only hyperimmune serum would cause adherence if the serum had been heated. The authors say that it seems probable that eosinophils in a non immune host may combat invading *D. viviparus* larvae in the presence of complement and that this killer cell activity would be enhanced by circulating antibody in an immune host.

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Phenol poisoning in a newborn lamb

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PHENOL poisoning in adult sheep following the use of phenolic dips has recently been reported (Veterinary Investigation Service 1980). Reported below is a case of phenol poisoning in a newborn lamb which resulted from the over-zealous use of a phenol containing disinfectant, recommended by the manufacturers for use at lambing time.

At 18.00 hours on day 1 the shepherd observed that a newly lambled Cheviot ewe was rejecting one of its twin lambs. To rectify this situation he placed the ewe and the lambs in a small pen and coated the rejected lamb with 200 to 300 ml Terebene sheep balsam (West Cumberland Farmers, Animal Health Department) which contains 3.7 per cent phenol w/w. A smaller quantity of this solution was applied to the ewe's nostrils.

At 08.00 hours on day 2 the lamb, which weighed 2.6 kg, was presented exhibiting convulsive flexion of the spine and with a rectal temperature of 33.6°C. The lamb was washed and dried, given 26 ml 20 per cent dextrose solution by intraperitoneal injection and placed in an incubator at 45°C. By 10.35 hours the rectal temperature had risen to 38.9°C and the lamb was removed from the incubator. The rectal temperature began to fall, however, so the lamb was returned to the incubator.

On day 3 the lamb was still unable to maintain normothermia outside the incubator and was unable to stand. A severe diarrhoea developed.

On day 4 the lamb was removed from the incubator and

was able to maintain normothermia when placed in a small pen heated to 20°C by means of an infra-red lamp. The lamb was weak and stood pressing its head into a corner of the pen. Muscular fasciculations were observed over the lamb's back.

On days 5 and 6 the diarrhoea and other symptoms gradually remitted and the lamb became stronger.

From day 7 onwards the lamb made an uneventful recovery and was eventually weaned at eight weeks of age at a body-weight of 15 kg.

From day 2 to day 6 the lamb was unable to suck from a bottle and was fed three times daily by stomach tube at a dose rate of 50 ml per kg. On days 2 and 3, cow colostrum was fed and from day 4 onwards a milk substitute was used (Ewebol orphan lamb food; BOCM Silcock). From day 2 to day 7 the lamb received 50 mg ampicillin trihydrate with 100 mg activated attapulgit orally twice daily (Penbritten oral doser; Beecham Animal Health). Blood samples were withdrawn from a jugular vein into heparinised syringes at the times indicated in Fig 1 (the first sample was taken before the glucose injection). The plasma was separated by centrifugation and stored at -20°C. The concentration of glucose was estimated according to the method of Trinder (1969) and the concentration of phenol was estimated using high performance liquid chromatography.

The plasma phenol concentration on presentation was 82 mg per litre (Fig 1). After two days the concentration fell to 5 mg per litre and on day 18 no phenol was detectable. The glucose level on presentation was 1.7 mM and thereafter remained between 1.7 and 9.8 mM.

Muscular convulsions and fasciculations, central nervous depression, diarrhoea due to local gut irritation and poor thermoregulatory ability are all reported symptoms of phenol poisoning (Leschke 1934, Radeleff 1970, Clarke and Clarke 1975). The observation of these symptoms in this lamb combined with the presence of substantial quantities of phenol in the circulation and the absence of hypoglycaemia, a common cause of hypothermia in newborn lambs (Eales and others 1980), supports the diagnosis of phenol poisoning.

Phenol is absorbed orally and via the skin (Barnes 1968, Clarke and Clarke 1975) and it would seem likely that both routes of absorption were operative in this case. It would appear likely that the presenting symptoms of the lamb were a direct result of the actions of phenol on nervous and muscle tissues. However the phenol was substantially cleared from the circulation by day 4 (Fig 1) and the symptoms exhibited from this time onwards were probably the result of tissue damage.

This case demonstrates that newborn lambs should not be exposed to excessive quantities of phenolic compounds but that intensive conservative treatment of affected animals can be effective.

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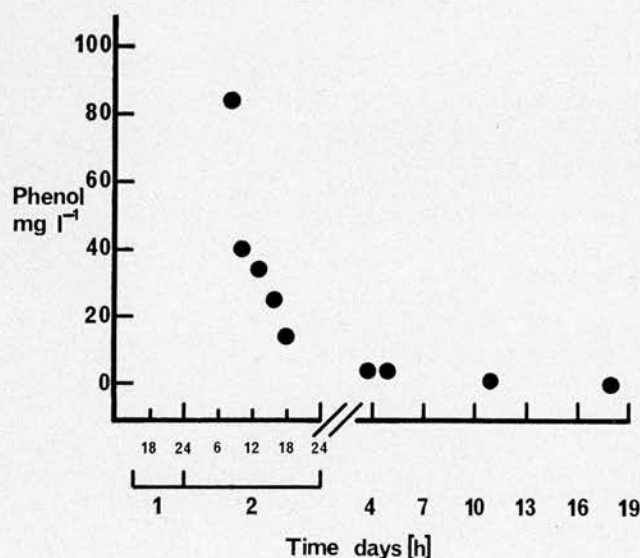


FIG 1: Plasma phenol concentrations in the lamb

Resuscitation of hypothermic lambs

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A technique was developed for the resuscitation of hypothermic newborn lambs. This technique consisted of three major components: the administration of glucose solution by intraperitoneal injection to lambs aged six hours or more in order to reverse hypoglycaemia (10 ml/kg of a 20 per cent solution); rewarming the lambs in air at 40°C; and careful attention to the nutrition and husbandry of the lambs after rewarming. The results indicate that careful application of this technique during lambing would considerably reduce the losses from hypothermia.

MARKED hypoglycaemia has been demonstrated as a characteristic of hypothermic lambs aged about six hours or more (Eales and others 1980, Eales and others 1982). During rewarming, these lambs commonly exhibited convulsive behaviour which was soon followed by death. However, the administration of glucose before rewarming prevented this by reversing the hypoglycaemia. A practical technique for the administration of glucose as part of a general regime for the resuscitation of hypothermic lambs has been developed. This regime, together with new techniques for rewarming and post resuscitation care, is described here.

Materials and methods

Resuscitation was attempted on 89 hypothermic lambs from two commercial farms, which were identified in the field by the shepherds on the basis of behaviour and appearance. Hypothermia, defined as a body temperature of 38°C or less, was confirmed by measurement of rectal temperature. The mean temperature of the lambs was $29.6 \pm 0.52^\circ\text{C}$ ($n = 89$).

Rewarming

The lambs were rewarmed in air at 40°C by means of a device (Fig 1) consisting of a small pen, measuring externally

7 feet (2.1m) square, made of horizontally laid straw bales, two bales high. The pen was divided horizontally into two chambers by a sheet of half inch (1.3cm) weld mesh and a steel tunnel was placed in a gap in the bottom layer of bales. Warm air was blown into the lower chamber from a 3 kW domestic fan heater positioned in the steel tunnel. A sheet of polythene was fitted over the top of the whole assembly to retain heat. The air temperature in the upper chamber was controlled either by adjusting the kilowatt setting on the heater, by adjusting the position of the heater in the tunnel or by cutting a small hole in the plastic sheet. A hypothermic lamb was dried with a towel and then placed on top of the weld mesh under the plastic sheet. When the rectal temperature rose to 38.0°C the lamb was removed.

Glucose injection

An intraperitoneal injection of glucose solution was given to 64 lambs before rewarming to investigate the efficacy of this for the reversal of hypoglycaemia (Eales and others 1982). A 50 ml syringe fitted with a 19 gauge 1 inch (2.6 cm) needle was used. The injection site was approximately ½ inch (1.3 cm) lateral to and 1 inch (2.6 cm) behind the umbilical stump. The needle was inserted at an angle of 45° to the skin surface and aimed at the lamb's rump. The effects of three doses of glucose were examined: 0.5 g/kg (10 ml/kg of a five per cent solution), 1.0 g/kg (10 ml/kg of a 10 per cent solution) and 2.0 g/kg (10 ml/kg of a 20 per cent solution).

Post resuscitation care

In the initial stages of the project no special measures were employed to care for the resuscitated lambs. They were either returned to the ewe or placed in a 'lamb bar' where they received limited attention. This resulted in a high rate of mortality, especially in lambs allocated to the lamb bar, and so a post resuscitation regime was progressively developed with the objective of improving survival. The main features of this regime were as follows

- (1) After removal from the warmer lambs were given 100 to 200 ml colostrum by stomach tube.
- (2) Lambs were placed in a cardboard box measuring approximately 2ft x 2ft x 2ft (0.6m). The box was bedded with newspaper and warmed by a 275 W infra-red lamp suspended 4 feet (1.3m) above the lamb. The box was destroyed after use.
- (3) Lambs were fed three times daily on cow colostrum or milk substitute. A bottle was used if the lamb could suck vigorously, if not it was fed by stomach tube at a dose rate of 50 ml/kg/feed.
- (4) Oral antibiotic, 1 ml containing 50 g ampicillin trihydrate and 100 mg activated attapulgit (Penbritin Oral doser; Beecham) was given to the lamb twice daily.
- (5) Lambs were returned to the ewe as soon as they could stand and suck vigorously.
- (6) If for any reason the lamb could not be returned to the ewe, but was physically strong and able to suck, it was removed to a lamb bar after two days where care was taken to ensure that good nutrition continued. Any lamb which was weak or showing signs of disease was retained in the small pen until fit.



FIG 1: Warming device for hypothermic lambs with plastic sheet and blower heater

Post resuscitation progress

The progress of resuscitated lambs was followed until weaning. In order to investigate this without the complication of maternal effects 10 resuscitated lambs together with nine healthy lambs which had not been hypothermic were artificially reared using a lamb bar. The lambs were weaned at a bodyweight of 15 kg. The health and growth rates of these two groups of lambs were monitored and compared.

Biochemistry

Heparinised blood samples were taken from the lambs as previously described (Eales and others 1982) before rewarming, one hour after the injection of glucose and again on removal from the warmer. The haematocrit value and plasma glucose concentration were estimated as described before (Eales and others 1982).

Pathology

Post mortem examinations were carried out on the 15 lambs previously described (Eales and others 1982).

Results*Resuscitation*

Successful resuscitation was defined as the lamb being alive, with a rectal temperature of 38°C, on removal from the warmer. Seventy-three lambs (82 per cent) were successfully resuscitated. Sixteen lambs died during resuscitation. In four cases death was attributed to extreme hypothermia (rectal temperature less than 20°C). In nine cases death was attributed to hypoglycaemia. These lambs exhibited convulsions which could be temporarily arrested by the intravenous injection of 5 ml 20 per cent glucose solution. One lamb was destroyed during rewarming because of gross abdominal distension and the cause of failure in two lambs was unknown. The mean time for resuscitation was 2.6 ± 0.18 h ($n = 67$).

Glucose injection

The effects of glucose injection on plasma glucose concentration and on haematocrit value are shown in Table 1. In the lambs aged less than six hours only the higher doses of glucose (1.0 and 2.0 g/kg) caused significant elevations of the plasma glucose level and there were no significant changes in haematocrit readings. However, in the lambs aged more than

six hours all doses of glucose caused a significant elevation of the plasma glucose level. The magnitude of the elevation was positively related to the dose of glucose. There were no significant changes in haematocrit values with the lower glucose doses but the highest dose rate (2.0 g/kg) did result in a small but significant elevation.

Post resuscitation survival

Of the 73 lambs which were successfully resuscitated, 48 (66 per cent) were alive at weaning at three to four months of age. The causes of death in the remaining 25 lambs were diagnosed as follows: uncomplicated starvation (six), trauma (two), inhalational pneumonia following bottle feeding (five), infections (four), patent ductus arteriosus and, or, incomplete expansion of the lungs (four) and causes unknown (four). In 22 of these lambs starvation was implicated as a contributory cause of death by an ante mortem plasma glucose level below 1.5 mM and, or, an absence of periodic acid-Schiff positive material in the liver indicating the exhaustion of hepatic glycogen reserves. The mean time between resuscitation and death was 6 ± 2 days ($n = 25$) but 18 of the 25 lambs died within three days of resuscitation.

Post resuscitation progress

Good health was maintained in all the artificially reared lambs. There were no significant differences between the hypothermic lambs and the normal lambs in birthweight, bodyweight at eight weeks of age or the age at weaning. The mean value for birthweight was 2.8 ± 0.21 kg, for bodyweight at eight weeks of age 16.9 ± 0.75 kg and for age at weaning 48 ± 1.8 days ($n = 19$).

Discussion

Resuscitation was successful for 73 of the 89 hypothermic lambs. Of the 16 lambs which died during resuscitation four died because of extreme hypothermia and it is doubtful if any treatment could have saved them. Nine lambs died during resuscitation because of hypoglycaemia which had not been effectively reversed. Subsequent experience showed that most of these lambs would have survived if an adequate dose of glucose had been administered before rewarming.

The warming device had a number of significant advantages over others, such as infra-red lamps. The required temperature was easy to monitor and control, the lamb was warmed on all sides, there was no risk of skin burns and hyperthermia was never observed. The use of an electric heater near straw bales could constitute a fire hazard although no problems were encountered. This potential

TABLE 1: Effects of an intraperitoneal injection of glucose solution on plasma glucose concentration and on haematocrit values in 64 hypothermic lambs

Age of lamb	Glucose dose (g/kg)	Number of lambs	Mean plasma concentration mM [*]			Change in haematocrit value during first hour after injection (%)
			On presentation	One hour after injection	On removal from warmer	
0 to 6 hours	0	6	2.2 ± 0.53	2.2 ± 0.48	2.3 ± 0.48	-1 ± 0.4
	0.5	21	3.9 ± 0.65	4.4 ± 0.70	$3.7 \pm 1.78^*$	-1 ± 0.6
	1.0 (2 lambs)	4	3.2 ± 1.26	$13.2 \pm 3.02^*$	10.2 ± 3.94	0 ± 1.0
	2.0 (2 lambs)					
Over 6 hours	0.5	15	0.5 ± 0.11	$1.6 \pm 0.30^\dagger$	1.2 ± 0.26	-1 ± 0.8
	1.0	8	0.5 ± 0.23	$2.7 \pm 0.60^\ddagger$	2.5 ± 1.00	0 ± 0.7
	2.0	10	0.8 ± 0.22	$5.2 \pm 1.02^\ddagger$	$6.6 \pm 1.35^*$	$2 \pm 0.8^*$

* $P < 0.05$

† $P < 0.01$

‡ $P < 0.001$

hazard could be practically eliminated by constructing the warmer with wood instead of straw.

The administration of glucose solution to lambs aged six hours or less, in which serious hypoglycaemia was uncommon, would seem to be unnecessary (Table 1). By contrast, the injection in lambs aged six hours or more, in which hypoglycaemia was common, was life saving. The results suggest that a dose of 2 g/kg is required for a sustained effect. This dose rate was associated with a small but significant elevation of the haematocrit value, presumably because water was drawn from the circulation into the peritoneal cavity. The maximum practical volume for injection is 50 ml and thus the maximum dilution which can be employed with a dose of 2 g/kg is 200 g/litre. In view of the increase in haematocrit which was observed when this solution was used it would seem imprudent to employ a more concentrated solution.

Of the 73 lambs which were successfully resuscitated 48 survived to weaning. Most of the 25 deaths occurred in the first three days after resuscitation and starvation was the cause or a contributory cause of death in 22 lambs. These findings demonstrate the importance of careful husbandry after resuscitation. Of the first 20 lambs successfully resuscitated only nine (45 per cent) survived to weaning but 17 (85 per cent) of the last 20 lambs survived. With the benefit of hindsight it would seem probable that many of the lambs which died after resuscitation in the early stages of the project

would have lived if they had been treated according to the post resuscitation regime which was finally developed. This simple regime guaranteed the lamb warmth, nutrition and protection from infection.

The diagnosis of inhalational pneumonia in five lambs which had been bottle fed after resuscitation highlights the danger of this type of feeding in weak lambs. The stomach tube should always be used in such cases.

The satisfactory post resuscitation progress of the lambs which were artificially reared demonstrates that lambs which have been hypothermic will develop normally providing they are given appropriate care. The present trend to increasing lambing percentages is likely to increase the number of lambs which become hypothermic. Prompt detection of hypothermia and appropriate measures of resuscitation should reduce mortality from this condition.

Acknowledgements.—We are grateful to all the staff at Cardrona Mains, Peebles and at Sourhope for their cheerful cooperation during lambing. We thank our many colleagues at the Moredun Research Institute and at the Hill Farming Research Organisation who have contributed to this study.

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Pregnancy diagnosis in dairy herds in England and Wales

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Veterinary Record (1982) **110**, 123-125

A survey of 1692 dairy farmers in England and Wales in 1979 revealed that 14.2 per cent had veterinary pregnancy diagnoses carried out on more than half their cows, 43.8 per cent on less than half and 42.0 per cent had none. The results showed a considerable increase in the use of pregnancy diagnosis since a survey in 1969. Farmers with larger herds tended to make more use of pregnancy diagnosis and there were also some regional differences. In 64.6 per cent of herds pregnancy diagnosis was carried out during the third and fourth months of pregnancy; 93.3 per cent of herds in which more than half the cows were diagnosed were examined before the fourth month. Owners of pedigree herds used pregnancy diagnosis more than commercial herd owners. The milk progesterone assay pregnancy test was used by 4.3 per cent of farmers on more than half their cows, and by a further 1.9 per cent of farmers on some cows. More than 75 per cent of farmers using the milk progesterone test also used veterinary pregnancy diagnosis in their herds.

REGULAR veterinary pregnancy diagnosis is accepted as an effective fertility management aid (Esslemont and Ellis 1975) and has been incorporated into herd fertility schemes by veterinary surgeons (Bloxxham 1980). Betteridge and Laing (1970) suggested that routine pregnancy diagnosis provided a means of identifying fertility problems in groups of animals at an early stage. The economics of early pregnancy diagnosis are now well quantified (Esslemont and Eddy 1977, Vaillancourt and others 1980) and confirm the value of this procedure despite possible hazards (Abbitt and others 1978, Paisley and others 1978).

Pregnancy testing by milk progesterone assay has been routinely available to dairy farmers in England and Wales

since 1975 (Booth and Holdsworth 1976, Booth 1979). The value of this early test (24 days after insemination) in combination with a later veterinary pregnancy test and the use of heat mount detectors on cows diagnosed not pregnant on either occasion, has been demonstrated (J. Boneschanscher and others 1980, personal communication).

A survey on the use of veterinary pregnancy diagnosis in 767 herds in England and Wales was carried out in 1969 by the Milk Marketing Board's Artificial Insemination Organisation (Milk Marketing Board 1969). This found that 9.8 per cent of herd owners had pregnancy diagnosis carried out on more than half their cows, 22.8 per cent had some diagnosis and 67.4 per cent had none. Farmers in the eastern region made the greatest use of pregnancy diagnosis, 30.4 per cent using it for over half the herd. The majority had tests carried out during the third month of pregnancy, although an appreciable proportion (14 per cent) of tests were after the fifth month.

A second survey carried out in 1979 in order to quantify the increased use of veterinary pregnancy diagnosis and to assess the impact of the milk progesterone assay method, is reported below.

Materials and methods

The survey was carried out by the two senior inseminators at each of the MMB's 86 artificial insemination centres and subcentres. Each inseminator completed a questionnaire on the first 10 dairy farms visited in August 1979. The questions covered the following points:

- (1) The size and type of herd
- (2) The proportion of the herd tested by veterinary pregnancy diagnosis

Farm Practice

Detection and treatment of hypothermia in newborn lambs

HYPOTHERMIA means an abnormally low body temperature (normal lamb 38.6 to 39.4°C). The condition is progressive in nature, once body temperature has fallen more than a few degrees it continues to fall until finally the lamb dies.

Lambs at risk

Although all lambs are liable to become hypothermic some are more susceptible than others. Lambs to which special attention should be paid include—

- (1) Lambs from ewes in poor condition.
- (2) Lambs from very young or very old ewes.
- (3) Twins and especially triplets.
- (4) Very small lambs.
- (5) Premature lambs.
- (6) Lambs which are limp and weak at birth.

Times of risk

There are two periods when lambs are most at risk from hypothermia—

Birth to five hours of age Hypothermia occurring at this time is generally due to excessive heat loss from the wet newborn lamb. This excessive heat loss may be compounded by a depression of heat production. The principal problem with these lambs is hypothermia itself and serious hypoglycaemia (a low blood glucose level) is uncommon. Colostrum helps the lamb through this period by increasing its heat production.

Ten hours to three days Hypothermia occurring at this time is generally due to depressed heat production as a consequence of starvation. The condition may be compounded by a high rate of heat loss due to lack of shelter and adverse climatic conditions. Lambs suffering this form of hypothermia commonly have two problems—hypothermia and hypoglycaemia.

Detection of hypothermia

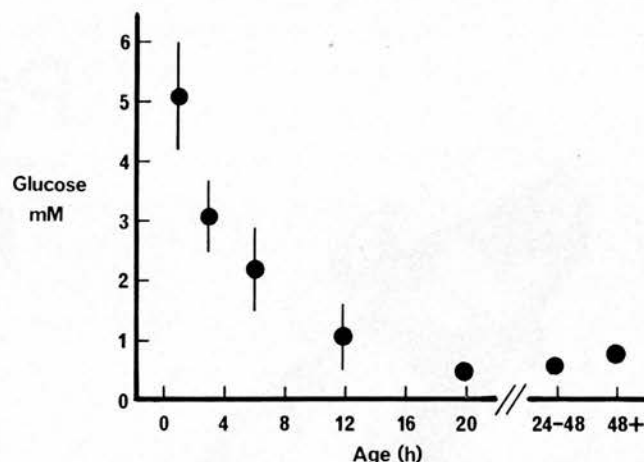
Profoundly hypothermic lambs are easily identified—they are comatose; but if treatment is to be effective early detection is essential. A thermometer must be used. The temperature of any lamb that appears at all weak should be taken. A number of instruments are available including the mercury clinical thermometer which reads only to 35°C , the sub-normal clinical thermometer which reads to 25°C , various electronic thermometers which normally read 0 – 100°C and the Moredun lamb thermometer designed specifically for shepherd use which indicates by means of flashing coloured lights whether the lamb

is not hypothermic ($>39^{\circ}\text{C}$), is moderately hypothermic (37 to 39°C) or is seriously hypothermic ($<37^{\circ}\text{C}$).

Treatment

The treatment required is related to both temperature and age.

Moderate hypothermia (37 to 39°C) in lambs of any age Conservative treatment is normally effective in these lambs. The lamb should be dried if wet, fed colostrum by stomach tube (50 ml/kg) and removed to shelter. There is no need to separate the lamb from the ewe providing she also can be removed to shelter.



The plasma concentration of glucose (mean \pm SEM) in 79 hypothermic lambs related to the ages at which they became hypothermic. The concentration in a healthy lamb should be 4 – 8 mM



A Moredun lamb thermometer

- 17 Central progressive retinal atrophy (PRD Type II) in the labrador retriever is primarily a disease of:
- cones
 - rods and cones
 - pigment epithelial cells
 - ganglion cells
 - bipolar cells
- 18 Which of the following clinical signs are consistent with a diagnosis of primary conjunctivitis?
- hyperaemia of conjunctival vessels
 - chemosis (conjunctival oedema)
 - iridodonesis
 - ocular discharge
 - deficiency of pre-corneal tear film
- 19 Which of these antibiotics are effective against *Pseudomonas* spp?
- chloramphenicol
 - polymyxin B
 - tetracyclines
 - neomycin
 - gentamycin
- 20 Fairly sudden onset (within 24 hours) of bilateral blindness in the dog may be due to:
- cataract
 - optic neuritis
 - retinal detachment
 - papilloedema
 - tentorial herniation

Answers on page 22



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Serious hypothermia (<37°C) in lambs aged less than five hours Serious hypoglycaemia is seldom a problem in these lambs. The lamb should be dried if wet and warmed in a 'warmer' as described below.

Serious hypothermia (<37°C) in lambs aged more than five hours Serious hypoglycaemia is commonly a problem in these lambs. If they are warmed in this state death from cerebral hypoglycaemia is likely. Treatment of these lambs consists of drying, an injection of glucose to reverse the hypoglycaemia followed by warming.

Techniques used in treatment

Drying The lamb must be dried before warming. Evaporation of water from the coat of a wet lamb during warming can result in a very high rate of heat loss which can considerably prolong the warming time. A towel should be used.

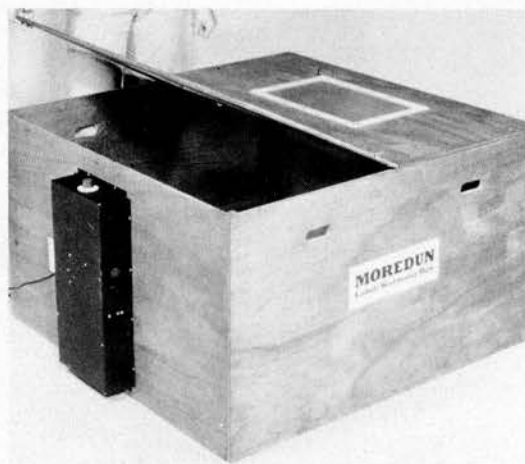
Glucose injection Hypoglycaemia is reversed by the intraperitoneal injection of glucose solution (10ml/kg of a 20 per cent solution). A sterile 50ml syringe fitted with a new 19g 1 inch needle is employed. The site for injection is 1 cm to the side and 2cm behind the umbilical stump. The injection is performed with the lamb held by the front legs. The needle is aimed at the lamb's rump (at an angle of 45°) and is fully inserted. Hygiene is of great importance in this procedure and parenteral antibiotic cover may be advisable.

Warming Lambs should be warmed in air at 37 to 40°C. This environment may be obtained by using a Moredun 'bale' warmer which is heated by a 3kw domestic blower heater, a Moredun lamb warming box or alternatively by using a home-made box of wooden construction based on the concept employed in the 'bale' warmer. Such a box should not be less than 1.5m square and 1m high otherwise there is a risk of overheating. This can cause hyperthermia which is rapidly fatal. Irrespective of the type of warmer used a thermometer should be placed near the lamb to ensure that the correct temperature is maintained. During warming the lamb's temperature should be

checked every 30 minutes. The lamb should be removed when 37°C is reached.

After-care

Good after-care is essential. Many lambs which are successfully warmed are later lost through poor after-care.



A Moredun lamb warming box



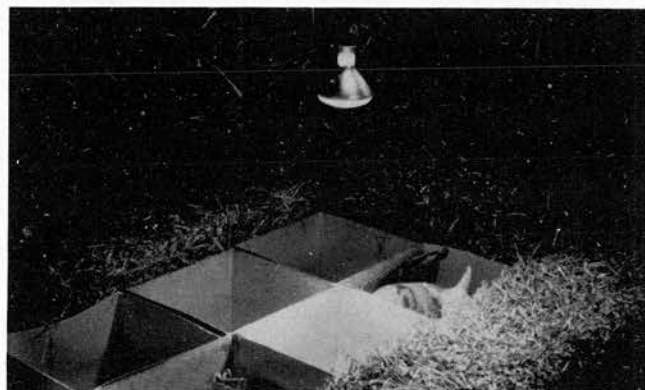
The site used for the glucose injection, 1cm to the side and 2cm behind the navel



The Moredun bale warmer in three stages of construction



A home-made warming box



An 'intensive care' unit for weak lambs

When removed from the warmer the lamb should be given colostrum by stomach tube (50ml/kg). If the lamb is strong and can suck vigorously it should be returned to its ewe in a sheltered pen. Care should be taken to ensure that the lamb is well fed and that hypothermia does not occur again. When twins are involved it is advisable to take both the lambs away while treatment is conducted and then return all the lambs to the ewe together.

A lamb which is weak after warming cannot be returned to a ewe. It will not suck and will very likely be injured. Weak lambs need a day or two of 'intensive care' for which there are three requirements. These are isolation, warmth and nutrition. Isolation is easily provided by housing the lamb in an individual cardboard box which is burnt after a single use. Warmth is provided by an infra-red lamp suspended about 1.3m above the lamb. Nutrition is assured by feeding the lamb three times daily with colostrum by stomach tube (50ml/kg per feed). A bottle should not be used since there is a serious risk of causing inhalational pneumonia and in addition tube-fed lambs are easier to return to a ewe. Any disease such as enteritis should be promptly treated. In some situations the routine administration of oral antibiotic to weak ex-hypothermic lambs may be advisable.

This resuscitation system is effective when a high standard of general husbandry is practised. A high level of hygiene is essential at all stages. All lambs which have recovered from hypothermia have suffered a severe insult including a marked depletion of their body energy resources. It is essential that these lambs are well fed and that further hypothermia is prevented.

This article describes the results of current work in the department of physiology at the Moredun Institute which are of interest to those involved in lambing and the resuscitation of hypothermic newborn lambs. Further information may be obtained from Mr F. A. Eales at the Moredun Research Institute.

Eye conditions in dogs and cats:

Answers to self-assessment test

- 1 b, c and d. CH is the most important diagnostic feature, tortuosity of retinal vessels and colobomatous defects may occur independent of CEA syndrome as may complications of retinal detachment and intraocular haemorrhage. Central/paracentral lipid dystrophy, originally described as part of CEA, now held to be separate entity
- 2 None of these. The cause is not always obvious. Antibiotics may delay healing. Recurrent epithelial erosion may represent a type of dystrophic recurrent erosion, in that attachment between epithelium and stroma is deficient because of anterior basement membrane abnormalities.
- 3 a, d and e
- 4 All of these
- 5 d
- 6 All of these. None of them would necessitate removal of membrana nictitans which is seldom indicated except, for example, with extensive neoplasia
- 7 All of these. Endothelial dystrophy invariably becomes bilateral. Corneal oedema may occur with severe iridocyclitis
- 8 All except d. Congenital means present at birth, which is not synonymous with inherited, although many congenital problems are also inherited
- 9 a, b, c and d
- 10 a, c and e
- 11 b and e, with d as a reasonably common occurrence later in the disease. Other ophthalmoscopic features include increased tapetal reflectivity, depigmentation of non-tapetal fundus, pallor of optic disc but *not* areas of pigmentation, an important distinguishing feature typical of central progressive retinal atrophy (PRD Type II)
- 12 a, b, c and e. Contraindicated in majority of situations where corneal epithelium not intact
- 13 All of these. May be some sparing of parasympathetic function. *Mild* ptosis is consequence of paralysis of levator palpebrae superioris
- 14 b, c and d. 'Cupping' of optic disc—a feature of chronic glaucoma. Aqueous flare may be a feature of anterior uveitis. Important to differentiate iritis/uveitis, keratitis and conjunctivitis from glaucoma
- 15 a, c and d. Also golden retriever, labrador retriever, standard poodle, old English sheepdog, Boston terrier, Staffordshire bull terrier, miniature schnauzer, West Highland white, beagle, alsatian and possibly others
- 16 All of these. Diabetes mellitus may produce lipaemia retinalis as a manifestation of hyperlipoproteinaemia; diabetic retinopathy not yet described in cat. Anaemia can result in pallid retinal vessels and retinal haemorrhages may also be present. Toxoplasmosis, FIP and TB provide variety of ocular signs including choroiditis, retinitis or combinations of the two
- 17 c. Primarily a pigment epithelial cell dystrophy. Other retinal layers secondarily involved
- 18 a, b and d. Deficiency of pre-corneal tear film may be a cause of secondary conjunctivitis. Iridodonesis (trembling of iris) pathognomonic of aphakia or lens luxation, the secondary glaucoma which may accompany the latter is frequently misdiagnosed as conjunctivitis
- 19 Order of efficacy—e, b and possibly d. Neomycin not totally reliable, chloramphenicol and tetracyclines, despite their broad spectrum, not usually effective against *Pseudomonas* spp
- 20 b, c, e and very occasionally a, in diabetes mellitus



HYPOTHERMIA IN THE YOUNG LAMB

INTRODUCTION

The Condition

Hypothermia occurs when body temperature falls below normal. It kills nearly one million lambs each year. Once a lamb's temperature has fallen more than 1–2°C it will continue to fall until the lamb dies, unless corrective measures are taken.

There are two periods when a lamb is most at risk (Figure 1):

1. Birth to five hours of age

At this age, a lamb becomes hypothermic when it loses heat faster than it can generate it. Energy reserves are not seriously depleted and such lambs have high blood levels of glucose (Figure 2). The best example is the wet newborn lamb which loses heat rapidly — especially if the ewe does not lick it dry or the weather is bad. The incidence of this type of hypothermia may be reduced by providing shelter which reduces heat loss and by ensuring that lambs quickly get colostrum which increases heat production.

2. Twelve hours of age and older

Starvation is the major cause of hypothermia in this period. The lamb becomes hypothermic because it cannot produce enough heat. Without a continuous supply of colostrum it soon burns up the energy reserves in its body and heat production falls from lack of fuel. These lambs have dangerously low blood glucose levels (Figure 2). The incidence of this type of hypothermia can be reduced by preventing hunger in newborn lambs. Good nutrition of the ewes before and after lambing should ensure strong lambs and a good milk supply.

Lambs at Risk

All lambs can suffer hypothermia but special attention should be given to:

1. Twins and triplets
2. Lambs from young or old ewes
3. Very small lambs
4. Lambs from ewes in poor condition
5. Lambs which are limp and weak at birth

New Technology

The resuscitation of hypothermic newborn lambs is not a new subject. However recent research work in this area has led to three developments which can now be put into practice. (a) Changes in electronics have enabled the production of a simple thermometer for **early** detection of the condition. (b) The finding that a low blood glucose level in older lambs can lead to death during warming but is preventable by an injection of glucose before rewarming. (c) The finding that warm air at 37–40°C is the ideal medium in which to warm hypothermic lambs and the development of practical methods for providing this environment. After considerable experience of their practical application a new system is proposed for the detection and treatment of hypothermia in newborn lambs.

Detection

Whilst severely hypothermic lambs may be obvious, slightly affected lambs are not — they may only appear a little weak. Successful resuscitation and return to the ewe depend on the early detection of the condition. A thermometer must be used. Even if a lamb arouses only the slightest suspicion its temperature should be taken. Practical experience has shown that the more the thermometer is used the lower are the lamb losses.

The Thermometer

The ideal instrument is the Moredun Lamb Thermometer* which is easy to use and immediately provides the shepherd with an indication of treatment needed by the use of flashing coloured lights. Alternative instruments include the mercury clinical thermometer which is difficult to use and easy to break, and electronic digital thermometers which at present can be mis-read and are not robust.

Using the Thermometer

The normal temperature of a lamb is 39–40°C. If the temperature of a suspect lamb is:

1. **MORE THAN 39°C** (green light on Moredun Lamb Thermometer): the lamb is not hypothermic. Some other disorder should be suspected. If in doubt consult your veterinary surgeon.
2. **BETWEEN 37 AND 39°C** (amber light) there is moderate hypothermia. Fairly simple treatment will save the lamb.
3. **BELOW 37°C** (red light) there is serious hypothermia. More radical treatment is required.

TREATMENT

Selection of Treatment

The type of treatment necessary is determined on the basis of temperature and age:

1. **37–39°C (amber), any age:** dry the lamb, feed it by stomach tube (details below) and give shelter along with the ewe.
2. **Below 37°C (red), less than 5 hours of age** (assume older if in doubt): dry the lamb and warm it.
3. **Below 37°C (red), but more than 5 hours of age:** dry the lamb, give it an injection of glucose (see below) to raise the blood glucose level, and warm it.

Drying the Lamb

The lamb must be dried before it is warmed. The easiest way to dry it is with a towel. If a wet lamb is placed in warm air it may lose more heat than it gains due to the evaporation of water from its coat. The net result is that the lamb gets colder. If an infra-red lamp is used the water may boil, causing very serious skin burns.

Glucose Injection

Consult your veterinary surgeon before attempting your first glucose injection because it is made into the belly (ie intraperitoneally). The technique is simple, safe and effective providing the correct procedure is

* produced to Moredun Institute design by Messrs Macam, 10 Kelvin Square, Livingstone.

followed. Equipment required:

- Sterile 50 ml syringes
- new 1" disposable needles (19 g)
- 40% glucose solution (500 ml bottles)
- Foot rot spray
- Electric kettle

The dose to be given to a lamb depends on its size:

- Large lamb — average single, about 5 kg : 50 ml 20% solution
- Medium lamb — average twin, about 3.5 kg : 35 ml 20% solution
- Small lamb — average triplet, about 2.5 kg : 25 ml 20% solution

The solution for injection should be prepared immediately before use.

Withdraw one half of the required dose from the bottle of 40% glucose. Dilute this with an equal volume of recently boiled water from the kettle. Shake syringe and ensure that the solution is at blood heat. If recently boiled water is used this will result automatically.

To perform the injection:

1. Hold the lamb by the front legs as shown in Figure 3.
2. Prepare the injection site (1 cm to the side and 2 cm behind navel) by spraying with foot rot spray.
3. Fully insert the needle (with syringe attached) at the injection site with the needle tip aimed towards the lamb's rump (at an angle of about 45°) (Figure 4).
4. Empty syringe and carefully withdraw. (The lamb may urinate during this procedure — this is not because the injection has gone into the bladder).
5. Dispose of needle and boil syringe before re-use.
6. Your veterinary surgeon may advise a precautionary injection of antibiotics for lambs which have been given glucose in this manner.

Warming

The ideal way to warm hypothermic lambs is in air at 37–40°C. This temperature should not be exceeded since overheating of the lamb is rapidly fatal. This warm environment may be obtained by making a 'bale' warmer (Figures 5 and 6) or by using the Moredun Lamb Warming Box* (Figure 7). The 'bale' warmer is cheap but is bulky, has a very slight fire risk and has poor temperature control. The more expensive Moredun Lamb Warming Box is compact, avoids all risk of fire and has automatic temperature control. A third alternative is a home-made wooden box based on the concept used in the 'bale warmer' (Figures 8 and 9). The dimensions of such a box should not be less than 1.5 m square and 1 m high otherwise there is a serious risk of overheating the lamb. Heating for bale or box warmers should be provided by means of a domestic fan-heater with 1 kW, 2 kW and 3 kW output settings and a **side** inlet port for safety reasons. Whatever type of warmer is employed it should stand on a layer of paper sacks to provide insulation. An ordinary household thermometer should be placed near to the lamb to be absolutely sure that the correct temperature is being maintained (37–40°C).

To warm the lamb, after it has been dried and given a glucose injection if necessary, place it in the top chamber of the warmer. The lamb's temperature should be checked every half hour and when its temperature exceeds 37°C (amber light on Moredun Lamb Thermometer) it should be removed. If the lamb is not removed at this stage there may be a risk of overheating. On removal the lamb should raise its temperature to 39°C very quickly by means of its own body heat.

A summary of the procedures is shown in table 1.

* made to Moredun Institute design by Messrs Macam, 10 Kelvin Square, Livingstone.

Table 1. Procedure for chilled lambs

Thermometer shows		Comment	Action required
39°C	Green	Temperature is normal or high	Seek another reason why the lamb is unwell
37–39°C	Yellow	Lamb is slightly chilled	Dry it Feed it Give shelter with ewe and her other lamb(s) Check again soon
Below 37°C	Red	Lamb is severely chilled	<p>If lamb is under 5 hours old Dry it Warm at 35–40°C until thermometer shows yellow or green (½–3 hours depending on initial state) Feed it</p> <p>If lamb is over 5 hours old As for lambs under 5 hours but in addition give glucose injection between drying and warming 50 ml for large lambs 35 ml for medium lambs 25 ml for small lambs (a 20% solution is used)</p>

POST-WARMING CARE

Occasionally lambs which are successfully warmed die later because of poor aftercare. The aims of post-warming care are to ensure that the lamb lives and to return it to a ewe (either its own or a foster ewe).

On removal from the warmer every lamb should be given a full feed by stomach tube (see next section for details).

The Strong Lamb

As soon as a lamb can stand and suck vigorously it should be returned to its dam in a sheltered pen. The lamb should be carefully observed to ensure that it is feeding (supplement by stomach tube if in doubt) and that hypothermia does not occur again. When only one lamb of a set of twins becomes hypothermic it is advisable to remove both lambs while treatment is being carried out. When the hypothermic lamb has been resuscitated both lambs should be placed in a small pen and both should be fed. After an hour or so when the resuscitated lamb will have regained its 'odour' both lambs can be returned to the ewe. A similar regime should be employed when triplets are involved.

The Weak Lamb

The weak lamb may require a day or so of 'intensive care' in order to build up strength if it is to be successfully returned to a ewe or fostered.

Such lambs have three requirements:

1. isolation to prevent cross-infection and to ensure individual monitoring and treatment,
2. warmth to prevent further hypothermia,
3. food to help the lamb build up its strength.

The first two requirements can be easily met by using a unit similar to that shown in Figure 8. Each lamb is allocated to its own cardboard box which is bedded with newspaper. After use the box is burnt. The infra-red lamp is suspended about 1.3 m above the lambs.

Consult your veterinary surgeon for advice on any other aspects of treating weak lambs especially if signs of disease appear eg scour.

The lambs should be returned to ewes as soon as they are strong enough. Care should be taken to ensure that they are well fed and do not become hypothermic again.

Occasionally, and particularly in lean ewes, the onset of milk flow can be delayed for up to 24 hours. Any further delay suggests a poor lactation so the ewe should be discarded and the lamb fostered or reared artificially.

FEEDING THE NEWBORN LAMB

Whenever it is necessary to feed a newborn lamb a stomach tube should be used. A bottle should not be used since with weak lambs there is a serious risk of causing pneumonia by the inhalation of milk. Tube-fed lambs are easier to foster and do not become 'bottle-orientated'. However it should be noted that it can be dangerous to feed severely chilled (hypothermic) lambs by stomach tube. In these weak and often semi-conscious lambs the tube can easily be passed into the windpipe and the animal drowned. If the feed is successfully placed in the stomach, absorption of nutrients may be very slow and there is a risk of regurgitation and inhalation of the feed into the lungs. Such lambs should be treated as outlined in the previous section and then fed.

The Equipment

A clean stomach tube and a clean 50 ml syringe are required. This equipment should be washed after each lamb and sterilised at least once daily by immersion in a dilute detergent/hypochlorite solution such as 'Domestos'.

Milk

The best food for newborn lambs is ewe colostrum. Where supplies are limited its use should be restricted to the first one or two feeds. It may be possible to accumulate a store by blending small quantities from a number of ewes. It should be dispersed into conveniently sized containers such as yoghurt cartons and deep-frozen. Surplus may be kept for the start of the following season. The colostrum should be warmed **gently** to blood heat before use. Fast defrosting renders the proteins less effective and so should be avoided.

Cow colostrum may have to be used because ewe colostrum is scarce. It too can be deep-frozen. It is a good substitute food but gives less disease protection. Lambs fed on it alone should therefore be given an injection of clostridial antiserum and vaccinated two weeks later. Both types of colostrum are superior to colostrum substitutes commonly recommended.

For lambs over 24 hours of age ewe milk replacer (reconstituted dried milk powder) is an excellent food but it is not a substitute for colostrum. Recently lambs were successfully reared on cow colostrum which has been allowed to sour naturally. A supply was obtained before lambing, kept in a cool place and used for three weeks straight out of the container.

Routine

Lambs should be fed three times daily eg 0700 h, 1500 h and 2300 h at the following dose rates:

Large lamb — average single, about 5 kg : 200 ml/feed

Medium lamb — average twin, about 3.5 kg : 150 ml/feed

Small lamb — average triplet, about 2.5 kg : 100 ml/feed

If it is practicable lambs can be fed more often — the quantity per feed should be reduced proportionately.

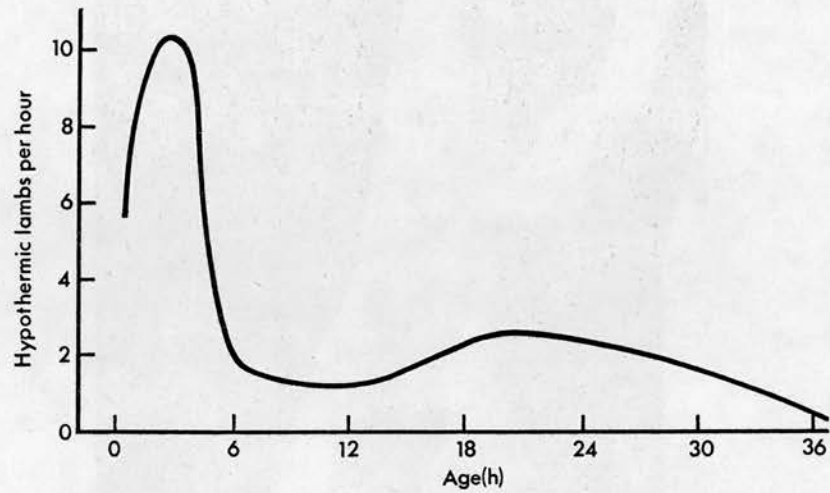
The stomach tube is often used to feed and invigorate a newborn lamb which is not hypothermic, but is slow to stand and suck. In this case 50–100 ml of colostrum should be given. However this is only enough food for 2–3 hours and if the lamb is not sucking well from the ewe at the end of this period further feeding is necessary.

Using the Stomach Tube

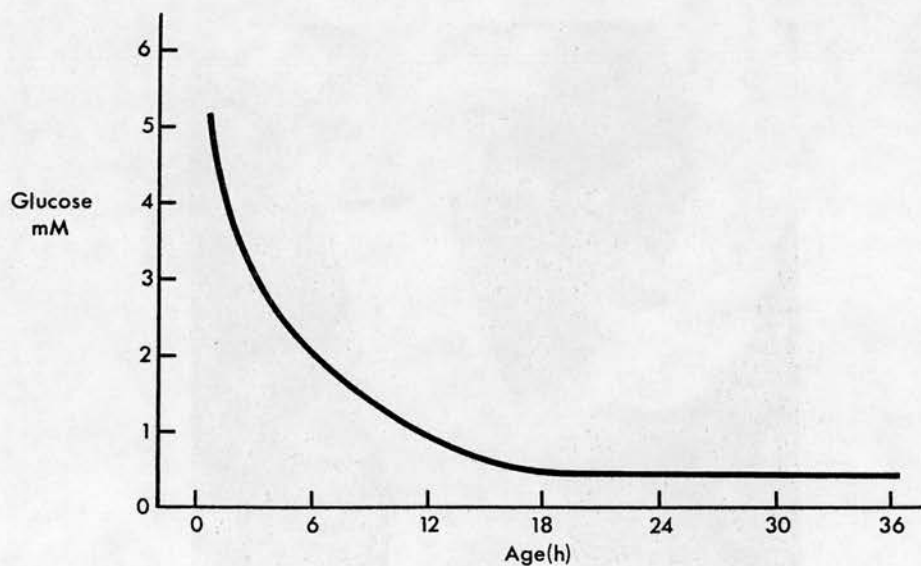
1. Sit comfortably on a stool or straw bale with the lamb on your lap (Figure 11).
2. Gently introduce a clean stomach tube (with no syringe attached) via the side of the mouth. No force is required. In a large lamb all but 2–5 cm of the tube can be easily introduced. If the lamb shows marked signs of discomfort withdraw the tube and start again.
3. Once the tube is in place observe the lamb (Figure 12). It should show no signs of distress and will probably chew the tube. This lack of discomfort proves that the tube is in the stomach.
4. Attach a syringe of colostrum to the end of tube. Empty the syringe slowly taking about 20 seconds (Figure 13). Remove the empty syringe and attach a full one. Repeat this process until the full feed has been given.
5. Finally remove both syringe and tube as a single unit and give the lamb freedom to move its head or cough if it so desires.
6. Wash and disinfect the tube and syringes.

Acknowledgements

We thank M G Christie for the development of the Moredun Lamb Thermometer, J Small for expert technical assistance and A W Speedy for constructive criticism.



1. The ages at which hypothermia occurred in a group of 79 affected lambs.



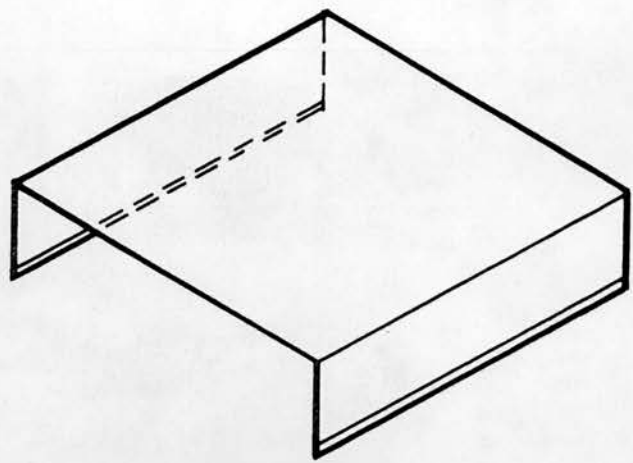
2. The plasma concentration of glucose in 89 hypothermic lambs related to the ages at which they became hypothermic. The concentration in a healthy lamb should be 4–8 mM.



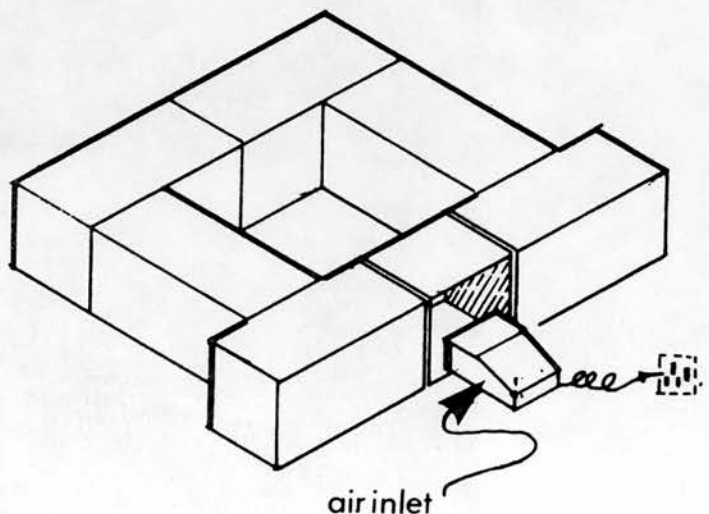
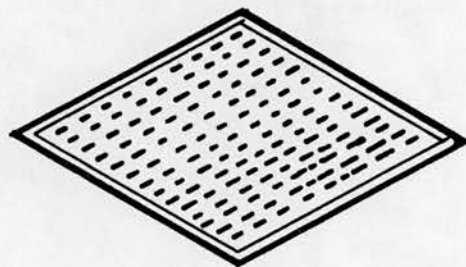
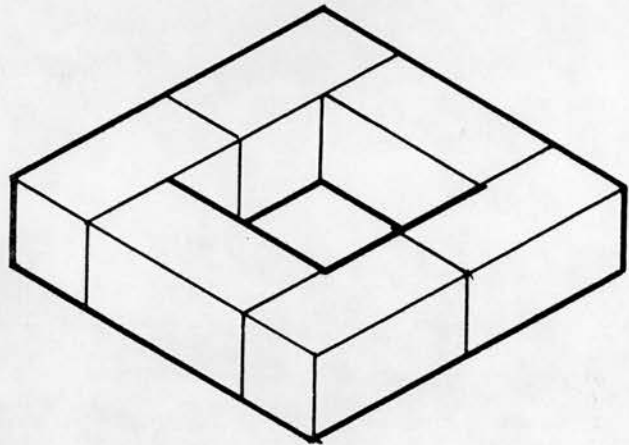
3. The site used for the glucose injection, 1 cm to the side and 2 cm behind the navel.



4. The glucose injection.



1. Consists of a sandwich of two decks each of six bales with a weldmesh (13 mm) or chicken netting tray between, on which the lambs are laid.
2. The bottom deck of bales should rest on a layer of paper sacks which should be burned after use.
3. The bales must be dry. Hay is preferable to straw.
4. The tray frame should be not less than 1.5 m × 1.5 m.
5. A metal-lined tunnel (375 mm high × 450 mm deep × 600 mm wide) for the heater should be placed in a gap made by parting the two bales at the front of the bottom deck.
6. Cover overall with 1,000 gauge clear plastic sheeting (2.15 m × 2.75 m) which can be adjusted for ventilation control. The sheet should overlap the bales at front and rear, where wood straps can be fixed to weigh it down.



5. The Moredun lamb warmer — bale model.



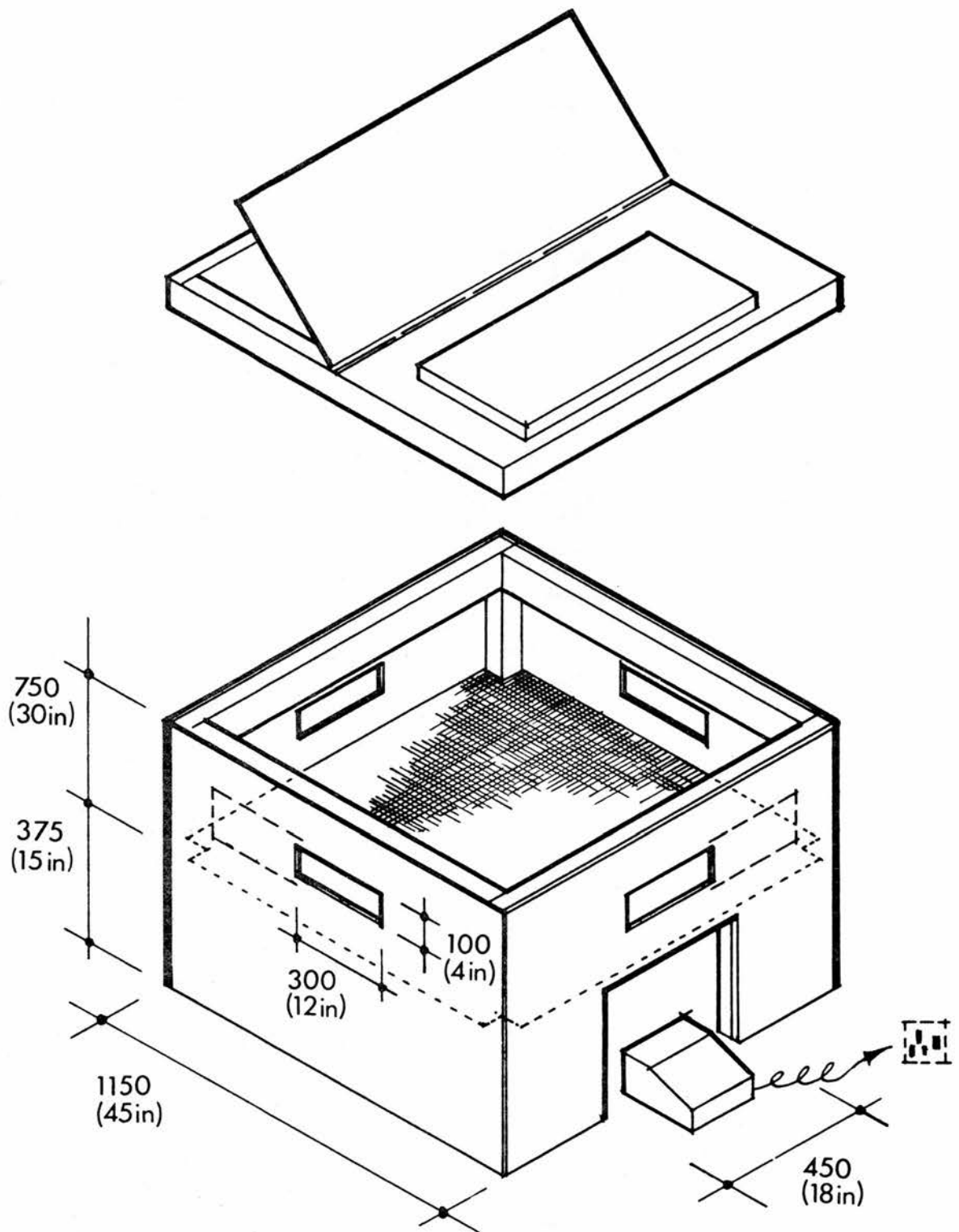
6. The bale warmer in three stages of construction.



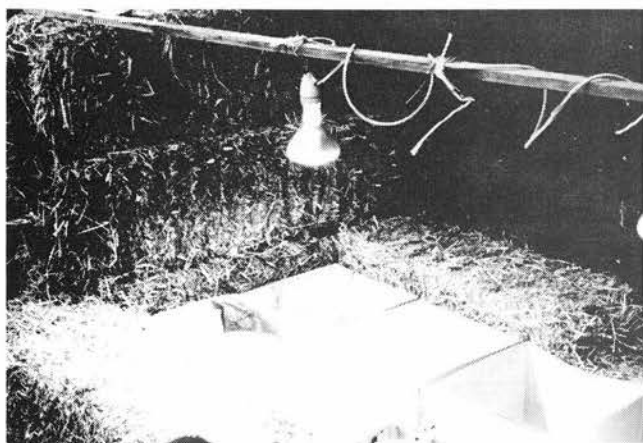
7. A Moredun Lamb Warming Box.



8. A home-made warming box.



1. Walls made of hardboard or plywood on timber framing.
2. Lid must open and must have a transparent inspection window.
3. False floor made of 13 mm ($\frac{1}{2}$ in) weldmesh.
4. Each of the four vents should have a sliding adjustable cover.



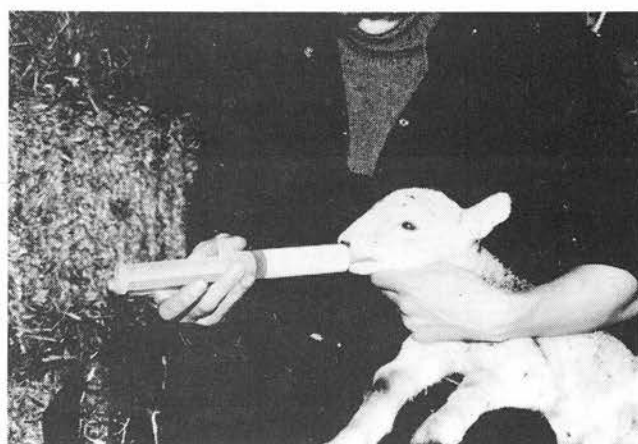
10. A post-warming care unit.



11. A comfortable position for stomach tube feeding.



12. The stomach tube in place.



13. Giving the feed.